Universidade Estadual de Londrina

ELISÂNGELA OLEGÁRIO DA SILVA

# EFEITOS DA FUMONISINA B1, DESOXINIVALENOL E ÁCIDO FÍTICO SOBRE EXPLANTES JEJUNAIS DE SUÍNOS:

AVALIAÇÃO MORFOLÓGICA, IMUNO-HISTOQUÍMICA, EXPRESSÃO DE CITOCINAS E ESTRESSE OXIDATIVO

Londrina
2018

ELISÂNGELA OLEGÁRIO DA SILVA

# EFEITOS DA FUMONISINA B1, DESOXINIVALENOL E ÁCIDO FÍTICO SOBRE EXPLANTES JEJUNAIS DE SUÍNOS: AVALIAÇÃO MORFOLÓGICA, IMUNO-HISTOQUÍMICA, EXPRESSÃO DE CITOCINAS E ESTRESSE OXIDATIVO

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutora.

Orientadora: Profª. Drª. Ana Paula Frederico Rodrigues Loureiro Bracarense

Londrina
2018

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Silva, Elisângela Olegário da.

Efeitos da Fumonisina B1, Desoxinivalenol e Ácido Fítico Sobre Explantes Jejunais de Suínos: avaliação morfológica, imuno-histoquímica, expressão de citocinas e estresse oxidativo / Elisângela Olegário da Silva. - Londrina, 2018.
136 f. : il.

Orientador: Profa. Dra. Ana Paula F.R.L. Bracarense.

Tese (Doutorado em Ciência Animal) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência Animal, 2018.
Inclui bibliografia.

1. Estresse oxidativo - Tese. 2. Micotoxinas - Tese. 3. Ácido fítico - Tese. 4. Suínos Tese. I. Bracarense, Profa. Dra. Ana Paula F.R.L. II. Universidade Estadual de Londrina.
Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência Animal. III. Título.

ELISÂNGELA OLEGÁRIO DA SILVA

# EFEITOS DA FUMONISINA B1, DESOXINIVALENOL E ÁCIDO FÍTICO SOBRE EXPLANTES JEJUNAIS DE SUÍNOS:
AVALIAÇÃO MORFOLÓGICA, IMUNO-HISTOQUÍMICA, EXPRESSÃO DE CITOCINAS E ESTRESSE OXIDATIVO

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutora.

**BANCA EXAMINADORA**

________________________________________
Orentadora: Profª. Drª. Ana Paula F.R.L. Bracarense
Universidade Estadual de Londrina – UEL

________________________________________
Prof. Dr. Caio Abércio da Silva
Universidade Estadual de Londrina – UEL

________________________________________
Prof. Dr. Emerson José Venâncio
Universidade Estadual de Londrina – UEL

________________________________________
Prof. Dr. Fernando Bittencourt Luciano
Pontifícia Universidade Católica – PUC

________________________________________
Profª. Drª. Joice Sifuentes dos Santos
Universidade Norte do Paraná – UNOPAR

Londrina, 23 de Julho de 2018.

O presente trabalho foi realizado no Laboratório de Patologia Animal/DMVP)UEL com colaboração:

- Laboratório de Inspeção de Produtos de Origem Animal (DMVP/UEL)
- Laboratório de Dor, Inflamação, Neuropatia e Câncer (CCB/UEL)
- Laboratório de Biologia Molecular de Microrganismos-Núcleo Integrado de Pesquisa (NIP) 5 (CCB/UEL)

Os recursos financeiros para o desenvovimento do projeto foram obtidos junto às agências e órgãos de fomento à pesquisa abaixo relacionados:

- Capes (Coordenadoria de Aperfeiçoamento de Pessoal de Ensino Superior)
- Fundação Araucária (Fundação de Amparo à Pesquisa do Estado do Paraná).

*Dedico este trabalho aos meus meninos Miguel e Arthur, tesouros preciosos que o Doutorado me deu de presente.*

# AGRADECIMENTOS

*"...E aprendi que se depende sempre*
*De tanta, muita, diferente gente*
*Toda pessoa sempre é as marcas*
*Das lições diárias de outrs tantas pessoas*
*É tão bonito quando a gente entende*
*Que a gente é tanta gente*
*Onde quer que a gente vá*
*É tão bonito quando a gente sente*
*Que nunca está sozinho*
*Por mais que pens estar..."*
*](Caminhos do Coração-Gonzaguinha)*

Agradeço a minha orientadora Ana Paula F.R.L. Bracarense pela oportunidade e confiança há 12 anos, e por me guiar no mundo da ciência até o presente momento. Pela paciência, ensinamentos, conselhos e encorajamento nessa trajetória. Foram muitos acertos, mas também erros e tropeços que fortaleceram nosso vínculo profissional, de amizade e respeito. Muito obrigada!

Aos professores Dr. Antônio Carlos Faria dos Reis e Selwyn Headley Arlington pelos ensinamentos e inspiração no mundo patológico.

Aos professores Dr. Waldiceu A. Verri Jr., Dra. Roberta Lemos Freire, Dr. Caio Abércio da Silva, Dr. Emerson José Venâncio, Dra. Joice Sifuentes dos Santos e Dr. Fernando Bittenourt Luciano pela colaboração no desenvolvimento deste projeto, e participação nas bancas de qualificação e defesa.

A amiga e "estatística sênior" Juliana Rubira Gerez, pela amizade,companheirismo e colaboração estatística, sem a qual não teria resultados, apenas números sem sentido.

A amiga e pós-graduanda Thalita E. S. de Oliveira pela ajuda, amizade e incentivo.

Aos amigos e colegas de profissão Karina Maria Basso, Luiz Daniel de Barros e Paulo Felipe Izique Goiozo pela amizade e parceria durante tantos anos.

Ao meu esposo Lucas, pela paciência, companheirismo, cumplicidade e carinho, suportanto as fases de estresse, se alegrando nos acertos e me animando e incentivando nos erros. Muito obrigada por me dar meus tesouros mais preciosos, Miguel e Arhur, presentes que vieram durante o desenvolvimento deste doutorado.

Ao Programa de Pós-graduação em Ciência Animal/UEL.

À Capes pela concessão da bolsa de Doutorado durante o desenvolvimento deste projeto.

À Funcação Araucária pelo auxílio financeiro.

A todos que estiveram presentes e contribuíram para a realização deste projeto. Muito obrigada!

*"Nenhum problema pode ser resolvido pelo mesmo estado de consciência*
*que o criou. É preciso ir mais longe. Eu penso várias vezes e nada descubro.*
*Deixo de pensar, mergulho em um grande silêncio e a verdade me é revelada."*
*(Albert Einstein)*

SILVA, Elisângela Olegário da. **Efeitos da fumonisina b1, desoxinivalenol e ácido fítico sobre explantes jejunais de suínos:** avaliação morfológica, imuno-histoquímica, expressão de citocinas e estresse oxidativo. 2018. 137 f. Tese (Doutorado em Ciência Animal)- Universidade Estadual de Londrina, Londrina, 2018.

## RESUMO

O objetivo do presente estudo foi avaliar os efeitos tóxicos das micotoxinas desoxinivalenol (DON) e fumonisina B1 (FB1) sobre o intestino e investigar a ação moduladora do ácido fítico (IP6) por meio de um modelo *ex vivo,* analisando a morfologia, proliferação celular, apoptose, expressão de E-caderina e ciclooxigenase-2 (cox-2), estresse oxidativo e expressão de citocinas em jejuno de suínos. Para tal, no primeiro experimento, foram utilizados cinco suínos com 24 dias de idade, os quais foram eutanasiados, o jejuno foi excisado, os explantes colhidos (40 explantes/animal) com um punch de 8 mm de diâmetro e submetidos a oito tratamentos (seis explantes/tratamento) : controle (somente meio de cultura), IP6 5mM, FB1 70µM, DON 10 µM, DON 10 µM+ FB1 70 µM, DON 10µM + IP6 5 mM, FB1 70 µM+IP6 5 mM e DON 10 µM+ FB1 70 µM+IP6 5 mM, incubados durante quatro horas sob agitação constante em estufa a 37°C. Após esse período, três explantes de cada tratamento foram fixados em solução de formalina tamponada a 10% e processados para realização do exame histológico e imuno-histoquímico para os anticorpos anti-ki-67, anti-caspase-3, anti-E-caderina e anti-cicloxigenase-2. Para a análise do estresse oxidativo, três explantes foram congelados em nitrogênio líquido, armazenados a -80C° e posteriormente processados para a avaliação dos níveis de glutationa reduzida (GSH), substâncias reativas ao ácido tiobarbitúrico (TBARS) e capacidade da atividade antioxidante por meio dos testes de 2, 2–azinobis-3-etil–benzotiazolina-6-ácido sulfônico (ABTS) e poder de redução do ferro (FRAP). Alterações morfológicas, diminuição da expressão de E-caderina, aumento da proliferação celular, apoptose, expressão de cox-2 e ocorrência de estresse oxidativo foram mais evidentes nos explantes expostos aos tratamentos com DON. A presença de IP6 reduziu significativamente as alterações morfológicas, proliferação celular, apoptose, expressão de cox-2 e estresse oxidativo, e aumentou a expressão de E-caderina nos explantes jejunais expostos às micotoxinas. Para avaliação da expressão de citocinas e peptídeos antimicrobianos, um segundo experimento com metodologia similar ao primeiro foi realizado. Explantes jejunais foram colhidos (21 explantes/animal) e submetidos aos seguintes tratamentos (três explantes/tratamento): controle (somente meio de cultura), FB1 70µM, FB1 70 µM+IP6 2,5 mM, FB1 70 µM+IP6 5 mM, DON 10 µM, DON 10µM + IP6 2,5 mM e DON 10µM + IP6 5 mM. Após a incubação, os explantes foram congelados em nitrogênio liquido e armazenados a - 80°C para avaliação da expressão das citocinas pró-inflamatórias (IL-1β, IL-6, IL-8, IL-10, IFN-γ, TNF-α) e anti-inflamatórias (IL-10) e β-defensinas 1 (pBD-1) e 2 (pBD-2) por meio da reação em cadeia pela polimerase em tempo real. A exposição às micotoxinas induziu a alterações na expressão das citocinas e β-defensinas. A presença de IP6, principalmente na dose de 5mM modulou os efeitos das micotoxinas, tornando a expressão da resposta inflamatória e antimicrobiana similar aos explantes controle. Os resultados do presente estudo evidenciaram os efeitos tóxicos da FB1 e DON sobre o jejuno de suínos, assim como o efeito protetor do IP6 em modular as alterações morfológicas, imunológicas e do estresse oxidativo induzido por tais micotoxinas.

**Palavras-chave:** Citocinas. Estresse oxidativo. Micotoxinas. Morfologia. IP6. Suínos.

SILVA, Elisângela Olegário da. **Effects of fumonisin B1, deoxynivalenol and phytic acid on jejunal explants of swine: morphologic and immunohistochemistry evaluation, cytokines expression and oxidative stress.** 2018. 137f. Thesis (Doctor's Degree in Animal Science)- Universidade Estadual de Londrina,Londrina, 2018.

## ABSTRACT

The aim of the present study was to evaluate the toxic effects of mycotoxins fumonisin B1 (FB1) and deoxynivalenol (DON) on the intestine, and investigate the modulatory action of phytic acid (IP6) through of *ex vivo* model and assessment of morphology, cell proliferation, apoptosis, E-cadherin and cyclooxygenase-2 (cox-2) expression, oxidative stress and cytokines and antimicrobial peptides in jejune of swine. In the first experiment, five pigs 24-days-old were euthanized and the jejunal explants were collected (40 explants/animal) using a punch of 8 mm in diameter and submitted to eight treatments (six explants/treatment): control (culture medium), IP6 5mM, FB1 70µM, DON 10 µM, DON 10 µM+ FB1 70 µM, DON 10µM + IP6 5 mM, FB1 70 µM+IP6 5 mM and DON 10 µM+ FB1 70 µM+IP6 5 mM, and incubated at 37°C under orbital shaking. After the incubation period, three explants *per* treatment were fixed in 10% neutral buffered formalin and processed for the histological examination and immunohisotchemistry assay using antibodies against ki-67, cleaved caspase-3, e-cadherin and cox-2. Three explants were immediately frozen in liquid nitrogen and posteriorly stored at -80°C to the assessment of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) levels. Morphological changes, decrease of E-cadherin expression and increase of cell proliferation, apoptosis, cox-2 expression and oxidative stress were more evident in the explants exposed to DON treatments. The presence of IP6 significantly decreased the morphological changes, cell proliferation, apoptosis, cox-2 expression and oxidative stress, and increased the E-cadherin expression in the jejunal explants exposed to mycotoxins in all treatments. A second experiment with similar methodology was performed to evaluate the cytokines and antimicrobial peptides expression. The jejunal explants without serosa were collected (21 explants/animal) and submitted to treatments (three explants/treatment): control (medium culture), FB1 70µM, FB1 70 µM+IP6 2.5 mM, FB1 70 µM+IP6 5 mM, DON 10 µM, DON 10µM + IP6 2.5 mM and DON 10µM + IP6 5 mM. After incubation, the explants were frozen in liquid nitrogen and posteriorly stored at -80°C to the assessment of pro- (IL-1β, IL-6, IL-8, IL-10, IFN-γ, TNF-α) and anti-inflammatory (IL-10), and β-defensins 1 (pBD-1) and 2 (pBD-2) by the reverse transcription polymerase chain reaction quantitative real time assay. The exposition to mycotoxins induced to changes in the cytokines and β-defensins expression. The presence of IP6, mainly in the 5 mM concentration, modulated the mycotoxins effects, becoming the expression of inflammatory and antimicrobial response similar to the control explants. The results of present study evidenced the toxic effects of FB1 and DON on the swine jejune, and the protective effect of IP6 in modulate the morphological and immunological changes, and the oxidative stress induced by these mycotoxins.

**Keywords:** Citokines. Oxidative stress. Mycotoxins. Morphology. IP6. Swine.

## LISTA DE ILUSTRAÇÕES

### REVISÃO DE LITERATURA

**Intestinal epithelial barrier of swine: compounds, homeostasis and toxic effect of the mycotoxins**

**Figure 1 -** Components of maintenance, integrity, and defense associated with the IEB ........ 47

**Effects of phytic acid on intestine: *in vitro*, *ex vivo* and *in vivo* studies**

**Figure 1 -** Molecular structure and configuration of IP6 ........ 54

**Mycotoxins and oxidative stress: where are we?**

**Figure 1 -** Summary of the intracellular lesions associated with oxidative stress induced by the main mycotoxins that contaminate food and feed ........ 69

**Figure 2 -** Summary of the intracellular lesions associated with oxidative stress induced by the main mycotoxins that contaminate food and feed ........ 70

### ARTIGOS

**Phytic acid decreases the oxidative stress and the intestinal elsions induced by fumonisin B1 and deoxynivalenol in swine**

**Figure 1 -** Effects of DON, FB1 alone or associated and IP6 on histological morphology in jejunal explants ........ 111

**Figure 2 -** Mean goblet cells density on villi and crypt of jejunal explants ........ 112

**Figure 3 -** Effects of DON, FB1 alone or associated and IP6 on apoptosis (Ccasp-3) and cell proliferation (ki-67) in jejunal explants ........ 113

**Figure 4 -** Effects of DON, FB1 alone or associated and IP6 on the E-cadherin and Cox-2 expression in jejunal explants ........ 114

## LISTA DE TABELAS

### REVISÃO DE LITERATURA

**Intestinal epithelial barrier of swine: compounds, homeostasis and toxic effect of the mycotoxins**

**Table 1 -** Toxic effects of mycotoxins AFB1, FB1, OTA, PAT and ZEN on the IEB compounds of pigs. .......... 48

**Table 2 -** Toxic effects of mycotoxins trichothecens (DON, NIV and T2-toxin) on the IEB compounds of pigs .......... 50

**Effects of phytic acid on intestine: *in vitro*, *ex vivo* and *in vivo* studies**

**Table 1** - Protective effects of IP6 on intestinal tissue in pathological conditions .......... 56

**Mycotoxins and oxidative stress: where are we?**

**Table 1 -** Effects of antioxidant vitamins A, C and E in mycotoxins studies .......... 74

**Table 2** - Effects of antioxidant food compounds (crocin and curcumin) in mycotoxins studies .......... 78

**Table 3** - Effects of antioxidant compounds (green tea/catechin/lycopene and phytic acid) in mycotoxins studies .......... 79

**ARTIGOS**

**Phytic acid decreases the oxidative stress and the intestinal elsions induced by fumonisin B1 and deoxynivalenol in swine**

**Table 1 -** Effect of DON, FB1 and IP6 on the oxidative stress in jejunal explants of swine .......... 110

**Phytic acid modulates the immunological response of cytokines and β-defensins in porcine intestine exposed to deoxynivalenol and fumonisin b1**

**Table 1 -** Nucleotide sequence of primers for real-time PCR .......... 113

**Table 2 -** Effect of IP6 on the mRNA expression of cytokines in swine jejunum exposed to DON and FB1 .......... 113

# SUMÁRIO


1 **INTRODUÇÃO** ........ 17

Referências ........ 18

2 **REVISÃO DE LITERATURA** ........ 20

2.1 INTESTINAL EPITHELIAL BARRIER OF SWINE: COMPOUNDS, HOMEOSTASIS AND TOXIC EFFECT OF THE MYCOTOXINS ........ 20

**Summary** ........ 20

**Introduction** ........ 20

**Compounds of maintenance, integrity and defense of the intestinal epithelial barrier** ........ 21

Intestinal epithelial cells (IEC) ........ 22

Intercellular junction protein ........ 22

Antioxidant defense mechanism ........ 23

Cytokines, chemokines and defensins ........ 24

Mucus layer ........ 25

Microbiota and lamina propria components ........ 26

***In vitro* and *ex vivo* models** ........ 26

**Mycotoxins** ........ 27

*Aflatoxin B1 (AFB1)* ........ 27

*Fumonisin B1 (FB1)* ........ 28

*Ochratoxin A (OTA)* ........ 29

*Patulin (PAT)* ........ 29

*Zearalenone (ZEN)* ........ 30

*Trichotehecenes* ........ 30

Deoxynivalenol (DON) ........ 31

Nivalenol (NIV) ........ 31

T-2 toxin ........ 32

**Conclusion and future approach** ........ 32

**Conflict of interest** ........ 32

**References** ........ 33

2.2 EFFECTS OF PHYTIC ACID ON INTESTINE: IN VITRO, EX VIVO AND IN VIVO STUDIES ........ 53
**Introduction** ........ 53
**Chemical, biological properties and intracellular functions** ........ 53
**Intestinal effect of IP6** ........ 54
Modulation of morphological changes ........ 55
Modulation of cell proliferation and apoptosis ........ 56
Antioxidant effects and modulation of immunological response ........ 57
Effects of IP6 on P-glycoprotein efflux ........ 58
**Conclusion** ........ 58
**References** ........ 59
2.3 MYCOTOXINS AND OXIDATIVE STRESS: WHERE ARE WE? ........ 65
**Abstract** ........ 65
**Introduction** ........ 66
**Oxidative stress: physiological control and damage caused by overproduction of free radicals** ........ 66
Generation of oxidative stress, free radicals, and damage to DNA, proteins and lipids ........ 67
**Response to mycotoxins and oxidative stress: interaction in *in vitro*, *in vivo* and *ex vivo* models** ........ 68
Aflatoxin B1 ........ 68
Deoxynivalenol ........ 70
Nivalenol ........ 71
T-2 toxin ........ 71
Fumonisin B1 ........ 72
Ochratoxin A ........ 72
Patulin ........ 73
Zearalenone ........ 73
**Antioxidants and mycotoxins: does a protective effect exist?** ........ 73
Vitamins ........ 74
Flavanoids ........ 76
Crocin, curcumin, green tea, lycopene and phytic acid ........ 77
L-carnitine ........ 77
Melatonin ........ 77

Minerals ........ 78
Mixtures ........ 79
**Conclusions** ........ 80
**References** ........ 80

**3 OBJETIVOS** ........ 89
Objetivo geral ........ 89
Objetivos específicos ........ 89

**4 ARTIGO 1** ........ 90
PHYTIC ACID DECREASES THE OXIDATIVE STRESS AND THE INTESTINAL LESIONS INDUCED BY FUMONISIN B1 NAD DEOXYNIVALENOL IN SWINE ........ 90
**Abstract** ........ 90
**Introduction** ........ 91
**Material and methods** ........ 92
*Animals and reagents (FB1, DON and phytic acid)* ........ 92
*Ex vivo experimental model* ........ 93
*Histological and imunohistochemical assesment* ........ 94
*GSH levels measurement* ........ 95
*Lipid peroxidation measurement (TBARS)* ........ 95
*ABTS and FRAP assays* ........ 96
*Statistical analysis* ........ 96
**Results** ........ 97
*Morphology assessment* ........ 97
*Caspase-3, Ki-67, E-cadherin and Cox-2 expression* ........ 98
*Oxidative stress evaluation* ........ 99
**Discussion** ........ 101
**Conclusion** ........ 105
**Acknowledgement** ........ 105
**References** ........ 105

5 **ARTIGO 2** ..... 115
PHYTIC ACID MODULATES THE IMMUNOLOGICAL RESPONSE OF CYTOKINES AND Β- DEFENSINS IN PORCINE INTESTINAL EXPOSED TO DEOXYNIVALENOL AND FUMONISIN B1 ..... 115
**Abstract** ..... 115
**Introduction** ..... 116
**Material and methods** ..... 118
*Animals* ..... 118
*Phytic acid* ..... 119
*DON and FB1 mycotoxins* ..... 119
*Jejunal explants technique* ..... 119
*Determination of the expression of mRNA encoding for cytokines by real-time PCR* ..... 120
*Statistical analysis* ..... 120
**Results** ..... 121
**Discussion** ..... 122
**Acknowledgements** ..... 126
**References** ..... 126

6 **CONCLUSÕES** ..... 134
**ANEXO** ..... 135
ANEXO A – Comissão de Ética no uso de Animais (2014) ..... 136

# 1 INTRODUÇÃO

As micotoxinas são metabólitos secundários de fungos que frequentemente são contaminantes presentes em cereais utilizados na alimentação humana e animal, comprometendo a saúde e gerando perdas econômicas que ultrapassam os 900 milhões de dólares por ano (DESJARDINS et al., 2003; WU et al., 2014). Devido a condições climáticas, produção e armazenagem dos grãos, a exposição às micotoxinas é inevitável, portanto, há uma necessidade crescente de estudos que objetivem prevenir ou mitigar seus efeitos tóxicos. Neste contexto, pesquisas com antioxidantes naturais têm evidenciado efeito benéfico e protetor contra a ação tóxica das micotoxinas em diferentes órgãos e espécies animais (ABIDIN et al., 2013; ABDEL-HAMID; FIRGANY, 2015; BEN SALEM et al., 2015).

O ácido fítico (IP6) é um antioxidante natural encontrado principalmente em cereais e legumes, cuja inibição da reação de Fenton e da produção de radicais livres, apresenta efeitos benéficos na prevenção e terapêutica de doenças degenerativas, metabólicas, inflamatórias e neoplásicas (ANEKONDA et al., 2011; KAPRAL et al., 2012). Estudos prévios *in vitro* (PACHECO et al., 2012) e *ex vivo* (SILVA et al., 2014) demonstraram efeitos protetores do IP6 sobre as alterações morfológicas induzidas pelas micotoxinas no intestino.

O trato gastrointestinal é uma importante barreira física e imunológica que protege o organismo contra a infecção de patógenos e à ação de substâncias tóxicas como as micotoxinas que são veiculados principalmente por meio dos alimentos (BOUHET; OSWALD, 2003). No entanto, mesmo com um complexo sistema de defesa, o intestino é um dos principais alvos dos efeitos tóxicos de diversas micotoxinas, com destaque para fumonisina B1 (FB1) e desoxinivalenol (DON), induzindo a alterações morfológicas e imunológicas que comprometem a homeostase e saúde intestinal.

Estudos evidenciaram que as alterações celulares desencadeadas pelas micotoxinas estão associadas a indução do estresse oxidativo com consequentes danos a síntese proteica, resposta inflamatória, lesão mitocondrial e ativação da apoptose (DEL REGNO et a., 2015; COSTA et al., 2016; LIU ; LU et al., 2017). A maioria dos estudos com este foco foi realizada *in vitro* e aborda o estresse oxidativo em rim e fígado expostos às micotoxinas. No entanto, a associação entre a indução do estresse oxidativo e os efeitos tóxicos no tecido intestinal não são conhecidos. Diante disto, os estudos *ex vivo* são importantes modelos pois preservam a morfologia tecidual e todos seus componentes de modo similar a modelos *in*

*vivo*, de modo a otimizar e minimizar uso de animais nos experimentos (RANDAL et al., 2011). Portanto, o objetivo do presente estudo foi avaliar os efeitos tóxicos das micotoxinas FB1 e DON e a ação modulatória do IP6 em explantes jejunais de suínos.

**Referências**

ABDEL-HAMID, A.A.M. FIRGANY, A.E.-D.L. Vitamin E supplementation ameliorates aflatoxina B1-induced nephrotoxicity in rats. **Acta Histochemica**, v. 117, p. 767-779, 2015.

ABIDIN, Z.; KHAN, M.Z.; KHATOON, A.; SALEEMI, M.K.; KHAN, A.; JAVED, I. Ameliorative effects of L-carnitine and vitamin E (α-tocopherol) on haematological and serum biochemical parameters in White Leghorn cockerels given ochratoxin A contaminated feed. **British Poultry Science**, v. 54, p. 471-477, 2013.

ANEKONDA, T.S.; WADSWORTH, T.L.; SABIN, R.; FRAHLER, K.; HARRIS, C.; PETRIKO, B.; RALLE, M.; WOLTJER, R.; QUINN, J.F. Phytic acid as a potential treatment for Alzheimer's pathology: evidence from animal and *in vitro* models**. Journal of Alzheimer's Diseases**, v. 23, p. 21-35, 2011.

BEN SALEM, I.; BOUSSABBEH, M.; HELALI, S.; ABID-ESSEFI, S.; BACHA, H. Protective effect of crocin against zearalenone-induced oxidative stress in liver and kidney of Balb/c mice. **Environmental Science and Pollution Research International**, v. 22, p. 190699-19076.

BOUHET, S.; OSWALD, I.P. The effects of mycotoxins, fungal food contaminats, on the intestinal epitehalial cell-derived innate immune response. **Veterinary Immunology and Immunopathology**, v. 108, p. 199-209, 2005.

COSTA, J.G.; SARAIVA,N.; GUERREIRO, P.S.; LOURO, H.; SILVA, M.J.; MIRANDA, J.P.; CASTRO, M.; BATINIC-HABERLE, I.; FERNANDES, A.S.; OLIVEIRA, N.G. Ochratoxin A-induced cytotoxicity, genotoxicity and reactive oxygen species in kidney cells: an integrative approach of complementary endpoints. **Food and Chemical Toxicology**, v.87, p. 65-76, 2016.

DEL REGNO, M.; ADESSO, S.; POPOLO, A.; QUARONI, A.; AUTORE, G.; SEVERINO, L.; MARZOCCO, S. Nivalenol induces oxidative stress and increases deoxynivalenol pro-oxidant effect in intestinal epithelial cells. **Toxicology and Applied Pharmacology**, v. 285, p. 118-127, 2015.

DESJARDINS, A.; MARAGOS, C.; NORRED, W.; PESTKA, J.; PHILLIPS, T.; VARDON, P.; WHITAKER, T.; WOOD, G.; VAN EGMOND, H. Mycotoxins: Risks in Plant, Animal, and Human System. **Council for Agricultural Science and Technology**. Ames, IA, USA, 2003.

KAPRAL, M.; WAWSZCUK, J.; JURZAZK, M.; HOLLER, A.; WEGLARZ, L. The effect of inositol hexaphosphato on the expressionof selected metalloproteinases and their tissue inhibitors in IL-1β-stimulated colon cancer cells. **International Journal of Colorectal Disease**, v. 27, p. 1419-1428, 2012.

LIU, Y.; WANG, W.J. Aflatoxin B1 impairs mitochondrial functions, activates ROS generation, induces apoptosis, and involves in Nrf2 signal pathway in primary broiler hepatocytes. **Animal Science Journal**, v. 87, p. 1490-1500, 2016.

LU, X.; ZHANG, E.; YIN, S.; FAN, L.; HU, H. Methylselenic acid prevents patulin-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress and inactivation of p53 and MAPKs. **Journal of Agricultural and Food Chemistry**, v. 65, p. 5299-5305, 2017.

PACHECO, G.D.; SILVA, C.A.; PINTON, P.; OSWALD, I.P.; BRACARENSE, A.P.F.R.L. Phytic acid protects porcine intestinal epithelial cells from deoxynivalenol (DON) cytotoxicity. **Experimental Toxicologic Pathology**, v. 64, p. 345-347, 2012.

RANDALL, K.J.; TURTON, J.; KOSTER, J.R. Explant culture of gastrointestinal tissue-a review of methods and applications. **Cell Biology**, v. 27, p. 267-284, 2011.

SILVA, E.O.; GEREZ, J.R.; DRAPE, T.C.; BRACARENSE, A.P.F.R.L. Phytic acid decreases deoxynivalenol and fumonisin B1-induced changes on swine jejunal explants. **Toxicology Reports,** v.1, p. 284-292, 2014.

WU, F.; GROOPMAN, J.D.; PESTKA, J.J. Public health impacts of foodborne mycotoxin. **Annual Review of Food Science and Technology**, v.5, p. 351-372.

# 2 REVISÃO DE LITERATURA

## 2.1 INTESTINAL EPITHELIAL BARRIER OF SWINE: COMPOUNDS, HOMEOSTASIS AND TOXIC EFFECT OF THE MYCOTOXINS[1]

**Summary**

The intestinal epithelial barrier (IEB) plays an important role in the intestinal protection against pathogens and toxic substances. The mycotoxins are common contaminants of feed worldwide and are considered a risk factor to swine health. The intestine of pigs is an important target to toxic effects of the major mycotoxins. This review addresses the main compounds associated to defense and maintenance of the intestinal epithelial barrier and summarizes the data about toxic effects of the major mycotoxins aflatoxin B1, fumonisin B1, ochratoxin, patulin, zearalenone, and trichothecenes (deoxynivalenol, nivalenol, and T-2 toxin) on the on the IEB integrity and homeostasis of pigs.

**Keywords:** intestinal homeostasis, fungal toxins, toxic effects, pig.

### Introduction

The intestinal mucosa has an important role in the absorption process of nutrients, water and electrolytes[1] and it is the first protection barrier against harmful microorganisms and toxic substances such as the mycotoxins.[2] The intestinal homeostasis and health depend on the balance between the microbiota, mucin production, compounds of intestinal epithelial cells and lamina propria elements.[3] An imbalance in these protective elements causes changes in the intestinal permeability and anormal immune response, inducing the malabsorption of

[1] Manuscrito editado de acordo com as normas de publicação da Jounal of Swine Health and Production (https://www.aasv.org)

nutrients and predispose to microbiological translocation, inflammatory and infectious diseases, and cancer development.[4]

Mycotoxins are secondary fungal metabolites often found as contaminants in almost all agricultural commodities worldwide and represent a risk for human and animal health.[5] More than 400 different mycotoxins have been isolated and chemically characterized, however, the aflatoxins, fumonisins, ochratoxins, trichothecenes, and zearalenone represents the major medical and agricultural concern.[6]

Swine is one the most sensitive species for the action mycotoxins and the intestine is an important target of their toxic effects inducing an imbalance of the mechanisms of the maintenance and integrity intestinal.[7,8] In addition, pigs are a useful model for translational research and clinical studies for improving human health due to the anatomical, physiological and immunological similarities.[9]

This review describes the compounds of intestinal epithelial barrier (IEB) involved in the maintenance of intestinal homeostasis and to summarize the toxic effects of the major mycotoxins on the IEB and their associated elements.

**Components of maintenance, integrity, and defense of the intestinal epithelial barrier**

The interaction between the components of intestinal epithelial barrier (IEB) intestinal such as epithelial cells (IEC), adherence and tight junctions, gap junctions, intracellular antioxidant mechanism, defensins, chemokines and cytokines expression, basal laminin, and mucus layer promoted health, homeostasis and microflora balance in the intestinal mucosa.[2,3,10] In addition, other mucosal components as Paneth cells, immunoglobulins expression and gut-associated lymphoid tissue with its intraepithelial lymphocytes (IEL) play an important role in the protection and maintenance of IEB [11] (Fig. 1).

Swine are sensitive to most mycotoxins and the intestinal mucosa is frequently a target of their toxic effects.[7, 12,13] Therefore, the knowledge of IEB functioning as the defense mechanism of intestinal tissue allows a better understanding of the toxic effects induced by the mycotoxins and their consequences to swine health.

*Intestinal Epithelial Cells (IEC)*

One layer of IEC (simple columnar epithelium) separates the intestinal lumen from subjacent tissue.[14] The epithelial integrity is fundamental in maintaining the selective barrier and protection of the submucosa against aggressive agents.[2] The enterocytes (absorptive cells) are the most abundant cell type and are completely renewed in approximately three to five days.[15] This turnover process is important to the regeneration of the intestinal mucosa and facilitates the exfoliation of infected enterocytes into the intestinal lumen.[16] The microfold cells (M cells) are specialized enterocytes present in the follicle-associated epithelium overlying Peyer's patches region and facilitate the delivery of luminal antigen to intestinal lymphoid organs.[14] The basal laminin subjacent to IEC, is an important structure involved in the molecules filtration and regulation of cell proliferation and differentiation.[17]

The IEC express a variety of pattern recognition receptors (PRRs) that recognize the pathogen-associated molecular patterns (PAMPs), produce interferon types I and III, antimicrobial peptides, cytokines and chemokines for recruitment and activation of immune system cells, and interacts with the mucus layer.[14,18] This epithelial transcriptional response is mediated by factor nuclear kappa B (NF-kappaB) and the mitogen-activated protein kinases (MAPKs) p38, JNK and ERK, and interferon regulatory factor 3 (IRF 3).[14]

*Intercellular junction protein*

The physical barrier composed by the IEC is an important intrinsic mechanism of immunity and their function is maintained through of intercellular structures including tight junctions (occludin, claudin, zonula occludens 1 and 2), adherence junctions (E-cadherin) and desmosomes.[2,19] These protein complexes provide an important physical barrier to

macromolecules and microorganisms, however, their structural organization is dynamic and inflammatory mediators as tumoral necrosis factor α (TNF- α), innate immune stimuli, enteric pathogens and food contaminants can influence the permeability, disrupting the junction proteins, and promoting bacterial invasion of intestinal mucosa.[14,20] Communications junctions (gap junctions) play an important role in direct cell to cell communication and coordination through exchange of electrical currents, small molecules, hormones and ions.[19]

In pigs, the measurement of transepithelial electrical resistance (TEER) of IEC can be used as an indicator of the degree of organization of the tight junctions and epithelial integrity.[21,22] In addition, immunohistochemistry assays can be used to evaluate the expression of junction proteins as E-cadherin and claudins.[7,23] Besides the TEER evaluation, the paracellular tracer flux assays are used to evaluate the integrity of intercellular junctions proteins in swine intestine through THE measurement of paracellular markers such as the fluorescent compounds [lucifer yellow (LY), horseradish peroxidase (HRP)] and fluorescently labeled compounds (fluorescein isothiocyanate-dextran (FITC-dextran) and fluorescein isothiocyanate-inulin (FITC-inulin) *in vitro*[21] and *ex vivo*[24]studies. *In vivo*, the paracellular tracer transport is measuremed by testing the presence of macromolecular tracers in the blood such as FITC-dextran and mannitol.[21,25]

*Antioxidant defense mechanism*

Reactive oxygen species (ROS) are normally produced in small quantities as consequence of endogenous reactions and are involved in normal cellular function such as maintenance of cell homeostasis, signal transduction, gene expression, and activation of receptors.[26] The cells present an antioxidant defense system able to inhibit the ROS generation, reduce oxidation and damage in DNA and in mitochondrial and cytoplasmatic membranes.[27] The antioxidant defense system in the intestine involve the thioredoxin system [thioredoxin (Trx) and thioredoxin reductases (TrxR)], vitamins C and E, glutathione (GSH) and the enzymatic antioxidants superoxide dismutase (SOD), glutathione peroxidase (GPx),

glutathione reductase (GR) and catalase (CAT).[28] The imbalance between the antioxidant defense and ROS production induce oxidative stress and chemical damage to DNA, proteins, and lipids, stimulating pathobiochemical mechanisms in the development of several intestinal diseases and cancer.[10,28,29]

Several methods have been developed to evaluate the cellular oxidative damage, radical species formation and intracellular antioxidant mechanisms.[30] Most of the assays used to evaluate the oxidative stress involve the measurement of GSH and antioxidant enzymes such as CAT, GR, GPx and SOD[30], lipid peroxidation [malondialdehyde level (MDA)][31], protein oxidation (concentration of carbonyl) and oxidative stress consequences such as DNA damage (alkaline comet (SCGE) assay, cytokinesis-block micronucleus (CBMN) assay and Fpg-modified comet assay).[32] Studies *in vitro*[32,33] and *in vivo*[34-36] have demonstrated that mycotoxins induce toxic effects through oxidative stress induction including ROS generation and a decrease in GSH and in endogenous antioxidants enzymes.

*Cytokines, chemokines, and defensins*

In the intestine, the pro-inflammatory cytokines IL-6, IL-8, IL-1β, IFN-γ, and TNF-α are produced by IEC (enterocytes) and play an important role in chemoatracting and activating inflammatory cells. These cytokines are up-regulated in microbial infections and cellular injury because chemical and toxic agents induce an inflammatory response.[37] On the other hand, the expression of anti-inflammatory cytokines such as IL-4 and IL-10 can protect intestinal tissues from inflammation inhibiting the activation of inflammatory cells, controlling the influx of immune cells and regulating cellular growth and differentiation .[38] In pigs, infectious agents such as *Salmonella* spp. and *Schistosoma japonicum* can upregulation the pro-inflammatory cytokine response.[39,40,41]

In pigs, β-defensins 1 and 2 are produced by enterocytes, goblet and Paneth cells[42,43] promoting activities are important against bacteria, fungi, enveloped virus and bacterial

toxins, and regulating commensal microbiota, besides performing functions associated to phagocytosis, neutrophil recruitment, and regulation of pro and anti-inflammatory cytokines.[44] In addition, IEC produce antibacterial peptides that is an effector molecule of cytotoxic T and NK cells and the development important activity against bacteria and fungi.[45]

The balance between the production of pro and anti-inflammatory cytokines, defensins and antibacterial peptides is essential in intestinal homeostasis resuting in protection against infectious agents, intestinal inflammation, cancer development and harmful effects of natural food contaminants such as mycotoxins.[8,38,44] In pigs, studies showed that the exposure to mycotoxins at intestinal level promoted changes in the cytokines and antimicrobial peptides expression in a dose-time-dependent manner inducing an inflammatory stimuli or an immunosuppressive *status*.[7,36,46,47]

*Mucus Layer*

The mucus layer is composed of mucins addressed with other proteins and lipids synthesized and secreted by goblet cells forming a continuous gel containing bicarbonate, that neutralizies pH at the epithelial surface.[2] Mucins are involved in gut physiology constituting a selective barrier permeable to nutrients.[48,49] In addition, the mucus layer present an interaction with antimicrobial peptides such as α-defensin 6, intestinal alkaline phosphatase and IgA, and promoting the fixation of commensal bacteria, detoxification of bacterial endotoxins, and the retention of bacteria and toxins within the lumen. Therefore contribute to the bacteria-free zone between the epithelial surface and intestinal lumen.[14,50]

In swine, dietary factors such as fiber, protein and anti-nutritional elements (tannin, lectin and protease inhibitor) influence the mucins production and the erosion of the mucus layer in the intestine.[51] Furthermore, enteric infectious disease agents nd ingestion of mycotoxins were associated to changes in the mucin production in the intestine of pigs.[7,22,52-54]

*Microbiota and components of lamina propria*

The intestinal microbiota presents important functions such as host defense by induction mucosal immune system and capacity to ferment components of the diet.[53] The microbial diversity and balance are infleuced by several factors such as diet composition, weaning and heat stress in pigs.[56,57] In addition, studies have demonstrated that mycotoxins exposition promoting changes into the microbiota and increase the translocation of pathogenic microorganisms.[58,59]

Due to the large number and diversity of enteric bacteria, it is inevitable that some commensal microorganisms may infiltrate the epithelial barrier and accessing the lamina propria.[3] However, there is a second line of defense that limits the microrganism ability to gain systemic access.[4] This defense mechanism is composed by immunoglobulins (IgA, IgG, and IgM), Paneth cells (antimicrobial secretion), dendritic cells, macrophages, natural killer cells (NK cells) and gut-associated lymphoid tissue with its intraepithelial lymphocytes (IELs).[4,60] The interaction between these compounds contributes with intestinal homeostasis and reinforces the IEB against bacterial and antigenic challenge.[4,53] In pigs, *in vitro* [62,63] and *in vivo*[63,64] studies reported the toxic effects of mycotoxin on microbiota balance and innate immunological system compounds as shown in Table 1 and 2.

***In vitro* and *ex vivo* models**

Most *in vitro* studies that have assessed the toxic effects of mycotoxins on the IEB was performed in human epithelial colorectal adenocarcinoma (Caco-2) culture cells line, due to features of these cells including spontaneous differentiation that result in polarized apical/mucosal and basolateral/serosal impermeable membranes and formation of the tight junction proteins between adjacent cells, result in a model that is structurally and functionally similar to IEC of the small intestine.[65,66] In pigs, the intestinal porcine epithelial cell lines 1 and 2 (IPEC-1 and IPEC-2) allow the evaluation of intracellular defense mechanism, protein

junction, and TEER, and to these features, have been used to investigate the effect of mycotoxins on the IEC. [67-69]

Intestinal explants have been used as an *ex vivo* model to evaluate the intestinal tissue of pigs.[70] The advantage of this model is that the explants maintain the intestinal architecture; therefore, the cells, structures and interactions can be evaluated. [66,71] This experimental model has been used in studies about the toxic effects of the mycotoxins on the intestine of swine.[23,72,73]

**Mycotoxins**

The mycotoxins aflatoxin B1, fumonisin B1, ochratoxin A, patulin, zearalenone and the trichothecenes (deoxynivalenol, nivalenol, and T-2 toxin) are the main contaminants of the food and feed worldwide due to their toxic effects on human and animal health, have been investigated extensively.[74] In pigs, the mycotoxins exposure can cause feed refusal, weight loss, anorexia, vomiting, skin dermatitis, hemorrhagic lesions, immunological, gastrointestinal and respiratory problems[5,8] promoting significant economic losses in the worldwide swine production. Several studies have demonstrated that the intestine of pigs [7,13,23,47] is a target for most mycotoxins, inducing morphological, physiological and immunological changes, and predisposing the animal to enteric infections. Some studies have suggested that the toxic effects of the mycotoxin on the intestine of pigs are dose and time-dependent.[70,73,75,76] The morphological, immunological, oxidative and lesional changes induced by mycotoxins are demonstrated in Table 1 and 2.

*Aflatoxin B1 (AFB1)*

Aflatoxins are produced mainly by *Aspergillus flavus* and *A. parasiticus* [77] and are natural contaminants of cereals, nuts, almonds, cottonseeds, and walnuts[6]. AFB1 is the most toxic and was associated with carcinogenic, hepatotoxic, nephrotoxic, genotoxic, immunotoxic, cardiotoxic and enterotoxic effects in humans and animals.[77] The toxicity of

AFB1 is associated mainly due to the binding of bioactivated AFB1-8,9-epoxide to cellular macromolecules, such as mitochondrial, nuclear nucleic acids and nucleoproteins, resulting in the cytotoxic effects.[78]

At intestinal level, studies have demonstrated that AFB1 decrease the TEER and claudin-3 (CLDN3), claudin-4 (CLDN4), and occludin (OCL) expression, affecting the intestinal permeability and morphology.[79,80] In pigs, the toxic effects of AFB1 were reported on the immunological system[81] , oocyte maturation [82], liver [83], and kidney. [84] The few studies on the intestinal exposure to AFB1 have demonstrated its effect on the cytokines expression and induction of oxidative stress as observed in Table 1.

*Fumonisin B1 (FB1)*

The fumonisins produced predominantly mainly by *Fusarium verticillioides* and *F. proliferatum,* widespread found on corn, wheat, barley and rice contaminants.[74] In animals, intoxication by FB1 causes leukoencephalomalacia in horses [85], and carcinogenic, hepatotoxic, nephrotoxic and embryotoxic effects in laboratory animals [86]. In addition, pulmonary edema, immunotoxic and enterotoxic effects were reported in pigs [23,87]. At the cellular level, FB1 inhibits ceramide synthase, blocking the synthesis of sphingolipids, a class of membrane lipids that plays an important role in cell signaling transduction pathways and cell growth, differentiation and death.[86] Ceramide synthase inhibition results reduced levels of ceramide and intracellular accumulation of sphingolipids (So) and sphinganine (Sa). These free sphingoid bases are proapoptotic, cytotoxic, growth inhibitors and immunotoxic.[88]

In swine, intoxication by FB1 was associated with toxic effects on immunological system[87], kidney[84], liver[89], lung [87] and intestine [7,23]. At intestinal level, the FB1 induce morphological changes in villi, goblet cells, and enterocytes, histopathological lesions, changes in inflammatory cytokines expression and lamina propria components, induce to oxidative stress and predisposes the animal to enteric infections (Table 1).

*Ochratoxin A (OTA)*

Ochratoxins are produced by filamentous fungi species *Aspergillus* and *Penicillium* and occurs in nature in three different isoforms as ochratoxin A, B, and C.[90] The ochratoxin A is the most pathogenic animals and is found in a wide variety of foods and feed, including cereals, meat, dried fruits, nuts, coffee, wine and beer.[91] Mammalian studies *in vitro*[90,92] and *in vivo* [93,94] have shown hepatotoxic, immunotoxic, enterotoxic, neurotoxic and teratogenic effects of OTA. The toxicity and carcinogenic mechanisms of OTA were associated with induction of oxidative stress[95], cell apoptosis [96], cell autophagy/mitophagy[35,97] and protein synthesis inhibition.[98]

In pigs, *in vitro* [61,99] and *in vivo* [35,100] studies have shown toxic effects of OTA-induced on the immunological system, kidney, and liver. Data about toxic effects on the intestinal are scarce (Table 1) and are associated with oxidative stress induced by OTA [101]. Most of the intestinal studies about OTA toxic effects were performed using Caco-2 cells and demonstrated a decrease in the TEER and protein expression of CLDN3 and CLDN4, an increase in permeability of HRP and 4 kDa FITC-dextran. [102-104]

*Patulin (PAT)*

Patulin is produced by several fungi species of the gender *Penicillium, Aspergillus, Paecilomyces*, and *Byssochlamys* and represents one of the main factors in losses of vegetables and fruits.[105] The toxic effects of PAT have been described *in vitro*[105-107], *in vivo* [108,109] and *ex vivo*[76] and were associated mainly to ROS generation, p53 protein, and cleaved caspase-3 activation. The rapid ROS generation observed in patulin toxicity is likely due to its electrophilic attack of the intracellular antioxidant enzymes containing sulfhydryl group as the glutathione[110]. At the intestinal level, *in vitro* studies using Caco-2 cells showed that PAT induced decrease of TEER and junctional proteins (CLDN4, OCLN and ZO-1) expression increase in paracellular permeability, translocation of commensal *Escherichia coli* and

expression of pro-inflammatory cytokines[104,106,111,112]. In pigs, *in vitro* studies demonstrated the toxic effects of PAT on lymphocytes [113] and in the renal cell line LLC-PK1 [114]. However, toxic effects on the swine IEB remain poorly understood as observed in Table 1.

*Zearalenone (ZEN)*

Zearalenone is produced by *Fusarium* fungi and is commonly found in unprocessed maize kernel.[5] ZEN and its metabolites (α-zearalenol [α-ZOL]; β-zearalenol [β-ZOL]) have a structural analogy to estrogen. The estrogenic activity of ZEN and its derivatives have been determined both *in vivo*.[115-118]

ZEN binds to estrogen receptors and causes alterations in the reproductive tract of humans, laboratory and domestic animals and was associated with decreased fertility, uterus enlargement, increased embryolethal resorptions, changes in the serum levels of progesterone and estradiol and endometrial adenocarcinoma.[5,116] In addition, intoxication by ZEN can to induce hepatocarcinoma, enterotoxic, immunotoxic and nephrotoxic effects.[36]

The swine is considered the animal species most sensitive to ZEN intoxication and its estrogenic activity was associated with alterations in fertility and reproduction.[119] In addition, a decrease in feed intake and body weight, immunological changes, kidney lesions and oxidative stress induction have been associated with intoxication by ZEN.[120] At intestinal level, the ZEN induces significant morphological and immunological changes, oxidative stress and ROS production and imbalance of the microbiota (Table 1).

*Trichothecenes*

Trichothecenes represent a large family of toxins produced by *Fusarium, Mycrothecium* and *Stachybotrys* fungi and are a potential threat to human and animal health due to the contamination of grains and cereals worldwide.[5] These families of mycotoxins are easily absorbed via the integumentary and gastrointestinal systems and have a rapid toxic effect on the immune system.[8] Deoxynivalenol, nivalenol and T-2 toxin are considered the

most important trichothecenes due to their widespread occurrence and potential risk for health and economic losses.[5]

Deoxynivalenol (DON)

Deoxynivalenol is a trichothecene type B predominantly produced by *Fusarium graminearum* and *F. culmorum* species contaminating basic grains such as wheat, maize, barley, and oats.[5] The exposure to DON has been associated with alterations of the intestinal, immune, endocrine and nervous systems in several animal species and humans.[121,122] At the molecular level, DON induce ribotoxic stress inhibiting the protein synthesis by binding to the ribosomal RNA peptidyltransferase site, inducing phosphorylation MAPKs, promoting apoptosis, inducing changes to the inflammatory response and decreasing the expression of cell adhesion proteins.[23,87,123]

In swine, the toxic effects induced by DON were reported mainly in the immunological system [87,124], kidney [120], liver [87], ovaries [125] and intestine [7]. Studies *in vivo* [7], *ex vivo*[21,23,72] and *in vitro*[126,127] have demonstrated that the intestine is the major tissue affected by DON. The changes induced by DON in the compounds of the intestinal barrier of pigs are described in Table 2.

Nivalenol (NIV)

Nivalenol is a trichothecene type B and generally is a biologically active metabolite of DON, present in agricultural commodities.[68] Studies *in vivo*[128], *in vitro* [129] and *ex vivo* [68] have been established that at the molecular level, NIV such as DON induces inhibition of protein, DNA, and RNA synthesis, mitochondrial damage, cell apoptosis, decreases cellular viability and modulates the inflammatory response mainly to ROS generation affecting mostly the gastrointestinal tract and organs of the immune system. In pigs, the toxic effects induced by NIV were reported in hematological and immunological systems[73,130] and liver [13].In the intestine, the changes promoted by the NIV exposure were more severe when compared to

other trichothecenes.[13,73,131] The toxic effects of NIV on the morphology, integrity and defense mechanism in the intestine are summarized in Table 2.

T-2 toxin

The T-2 toxin is classified as a trichothecene type A produced by several *Fusarium* species, mainly *Fusarium sporotrichiodes, F. poae and F. langsethiae* that is predominantly found in grains such as wheat, maize, rice, barley, oats and soybeans.[132] Studies demonstrated the T-2 toxin poisoning affect the gastrointestinal tract, kidney, liver, heart, nervous, skin, immunological and reproductive systems, and embryogenic development in humans and.[133-135] The T-2 toxin binds and inactivates the peptidyltransferase activity resulting in the inhibition of protein synthesis and disruption of the mitochondrial morphology, endoplasmic reticulum (ER) and other membranes.[136]

Toxic effects on the immunological system [137,138], liver [139], ovaries [140] and intestine[14,142] were reported in pigs. Table 2 summarizes the toxic effects induced by T2-toxin on IEB observed *in vivo, ex vivo* and *in vivo* studies.

## Conclusion and future approach

Several studies have demonstrated the toxic effects of mycotoxins on the intestine of pigs. However, due to the quantity and complexity of the mechanisms involved in the maintenance of integrity and homeostasis of IEB, more studies are necessary to elucidate the pathways and factors associated to intestinal changes and lesions induced by the mycotoxins. Furthermore, the knowledge of intestinal defense mechanisms and how mycotoxins affect their compounds allow the development of strategies to mitigate the harmful impact of the mycotoxins on pigs production.

## Conflict of interest

None reported.

**References**


1. Nedjfors P, Ekelund M, Jeppsson B, Westrom BR. Mucosal in vitro permeability in the intestinal tract of the pig, the rat, and man: species-and region-related differences. *Scand J Gastroenterol.* 2000; 5: 501-507.

2. Oswald IP. Role of intestinal epithelial cells in the innate immune defence of the pig intestine. *Vet Res.* 2006; 37: 359-368.

3. Duerr CU, Hornef MW. The mammalian intestinal epithelium as integral player in the establishment and maintenance of host-microbial homeostasis. *Sem Immunol.* 2012; 24: 25-35.

4. Zhou L, Sonnenberg GF. Essential immunologic orchestrators of intestinal homeostasis. *Sci Immunol.* 2018; e1605.

5. Escrivá L, Font G, Manyes L. In vivo toxicity studies of Fusarium mycotoxins in the last decade: a review. *Food Chem Toxicol.* 2015; 78: 185-206.

6. Wu F, Groopman JD, Pestka JJ. Public health impacts of foodborne mycotoxins. *Ann Rev Food Sci Technol.* 2014; 5: 351-372.

7. Bracarense A-PFL, Lucioli J, Grenier B, Pacheco GD, Moll W-D, Schatzmyr G, Oswald IP. Chronic ngestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. *B J Nutr.* 2012; 107: 1776-1786.

8. Pinton P, Oswald IP. Effect of deoxynivalenol and other Type B trichothecenes on the intestine: a review. *Toxins (Basel).* 2014; 6:1615-1643.

9. Braicu C, Cojocneanu-Petric R, Jurj A, Gulei D, Taranu I, Gras AM, Marin DE, Berindan-Neagoe I. Microarray based gene expression analysis of *sus scrofa* duodenum exposed to zearalenone: significance to human health. *BMC Genomics.* 2016; 17: 646.

10. Silva EO, Bracarense AFRL, Oswald IP. Mycotoxins and oxidative stress: where are we? *World Mycotoxin J.* 2018; 11:113-133.

11. Mukherjee S, Hooper LV. Antimicrobial defense of the intestine. *Immunity.* 2015; 42: 28-39.

12. Alassane-Kpembi I, Kolf-Clauw M, Gauthier T, Abrami R, Abiola F.A, Oswald IP, Puel O. New insights into mycotoxin mixtures: The toxicity of low doses of type B trichothecenes on intestinal epithelial cells is synergistic. *Toxicol Appl Pharm.* 2013; 272: 191-198.

13. Gerez JR, Pinton P, Callu P, Grosjean F, Oswald IP, Bracarense AP. Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. *Exp Toxicol Pathol.* 2015; 67: 89-98.

14. Zhang K, Hornef MW, Dupont A. The intestinal epithelium as guardian of gut barrier integrity. *Cell Microbiol.* 2015; 17: 1561-1569.

15. Radtke F, Clever H. Self-renewal and cancer of the gut: two sides of a coin. *Science.* 2005; 307: 1904-1909.

16. Sellin ME, Muller AA, Felmy B, Dolowschiak T, Diard M, Tardivel A, Maslowski KM, Hardt WD. Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict Salmonella replication in the intestinal mucosa. *Cell Host Microbe.* 2014; 16: 237-248.

17. Lepage M, Seltana A, Thibault MP, Tremblay E, Beaulieu JF. Knockdown of laminin α5 stimulates intestinal cell differentiation. *Biochem Biophys Res Commun.* 2018; 495:1510-1515.

18. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol.* 2015; 16: 343-353.

19. Bartle EI, Rao TC, Urner TM, Mattheyses AL. Bridging the gap: super-resolution microscopy of epithelial cell junctions. *Tissue Barriers.* 2018; 6:e1404189.

20. Bouhet S, Owald IP. The effects of Mycotoxins, fungal food contaminants, on the intestinal epithelial cell derived innate immune response. *Vet Immunol Immunopathol.* 2005; 108: 199-209.

21. Pinton P, Nougayrede JP, Del Rio JC, Moreno C, Marin DE, Ferrier L, Bracarense AP, Kolf-Clauw M, Oswald IP. The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. *Toxicol Appl Pharmacol.* 2009; 237:41–48.

22. Curry SM, Schwartz KJ, Yoon KJ, Gabler NK, Burrough ER. Effects of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity. *Vet Microbiol.* 2017; 211:58-66.

23. Silva EO, Gerez JR, Drape TC, Bracarense APFRL. Phytic acid decreases deoxynivalenol and fumonisin B1-induced changes on swine jejunal explants. *Toxicol Rep.* 2014; 1: 284-292.

24. Lalles JP, Lessard M, Boudry G. Intestinal barrier function is modulated by short-term exposure to fumonisin B1 in Ussing chambers. *Vet Res Commun.* 2009; 33:1039–1043.

25. Campbell NB, Ruaux CG, Shifflett DE, Steiner JM, Williams DA, Blikslager AT. Physiological concentrations of bile salts inhibit recovery of ischemic-injured porcine ileum. *Am J Physiol Gastrointest Liver Physiol.* 2014; 287: G399-G407.

26. Kumar S, Pandey AK. Free radicals: health implications and their mitigation by herbals. *Br J Med Med Res.* 2015; **7**: 438-457.

27. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free Radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007; 39: 44-84.

28. Diaz de Barboza G, Guizzardi S, Moine L, Tolosa de Talamoni N. Oxidative stress, antioxidants and intestinal calcium absorption. *World J Gastroenterol.* 2017; 23:2841-2853.

29. Assi M. The Differential Role of Reactive Oxygen Species in Early and Late Stages of Cancer. *Am J Physiol.* 2017; 313: R646-R653.

30. Yang HY, Lee TH. Antioxidant enzymes as redox-based biomarkers: a brief review. *BMB Reports.* 2015; 48: 200-208.

31. Zhou F, Sun W, Zhao M. Controlled formation of emulsion gels stabilized by salted myofibrillar protein under malondialdehyde (MDA)-induced oxidative stress. *J Agric Food Chem.* 2015; 63: 3766-3777.

32. Domijan AM, Gajski G, Jovanovic IN, Geric M, Garaj-Vrhovac V. In vitro genotoxicity of mycotoxin ochratoxin A and fumonisin B (1) could be prevented by sodium copper chlorophylin-implication to their genotoxic mechanism. *Food Chem.* 2015; 170: 455-462.

33. Wang H, Chen Y, Zhai N, Chen X, Gan F, Li H, Huang K. Ochratoxin A-induced apoptosis of IPEC-J2 cells through ROS-mediated mitochondrial permeability transition pore opening pathway. *J Agric Food Chem.* 2017; 65: 10630-10637.

34. Abbes S, Ben Salah-Abbes J, Jebali R, Younes RB, Queslati R. Interaction of aflatoxin $B_1$ and fumonisin $B_1$ in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. *J Immunotoxicol.* 2016; 13: 46-54

35. Gan F, Hou L, Zhou Y, Liu Y, Huang D, Chen X, Huang K. Effects of ochratoxin A on ER stress, MAPK signaling pathway and autophagy of kidney and spleen in pigs. *Environ Toxicol.* 2017; 32: 2277-2286.

36. Liu M, Zhu D, Guo T, Zhang Y, Shi B, Shan A, Chen Z. Toxicity of zearalenone on the intestines of pregnant sows and their offspring and alleviation with modified halloysite nanotubes. *J Sci Food Agric.* 2018; 98: 698-706.

37. Stadnyk AW. Intestinal epithelial cell as a source of inflammatory cytokines and chemokines. *Can J Gatroenterol.* 2002; 16: 241-246.

38. Szkaradkiewicz A, Marciniak R, Chudzicka-Strugala I, Wasileska A, Drews M, Majewski P, Karpiński T, Zwoździak B. Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. *Arch Immunol Ther Exp.* 2009; 57: 291-294.

39. Foss DL, Zilliox MJ, Murtaugh MP. Bacterially induced activation of interleukin-18 in porcine intestinal mucosa. *Vet Immunol Immunopathol.* 2001; 78: 263-277.

40. Oswald IP, Dozois CM, Barlagne R, Fournout S, Johansen MV, Bogh H. Cytokine mRNA expression in pigs infected with *Schistosoma japonicum*. *Parasitol.* 2001; 122: 299-307.

41. Splichal I, Trebichavcky I, Muneta y, Mori Y. Early cytokine response of gnotobiotic piglets to *Samonella enterica* serotype typhimurium. *Vet Res.* 2002; 33: 303-307.

42. Veldhuizen EJA, Koomen I, Ultee T, Van Dijk A, Haagsman HP. *Salmonella serovar* specific upregulation of porcine defensins 1 and 2 in a jejunal epithelial cell line. *Vet Microbiol.* 2009; 136: 69-75.

43. Wan ML-Y, Woo C-SJ, Allen KJ, Turner PC, El-Nezami H. Modulation of porcine β-defensins 1 and 2 upon individual and combined *Fusarium* toxin exposure in swine jejunal epithelial cell line. *Appl Environ Microbiol.* 2013; 79: 2225-2232.

44. Cobo ER, Chadee K. Antimicrobial human β-defensins in the colon and their role in infectious and non-infectious disease. *Pathogens.* 2013; 2: 177-192.

45. Andersson M, Gunne H, Agerberth B, Boman A, Bergman T, Sillard R, Jornvall H, mutt V, Olsson B, Wigzell H, Dagerlind A, Boman HG, Gudmundsson GH. NK-lysin, a novel effector peptide of cytotoxic T and NK cells. Structure and cDNA cloning of the porcine form, induction by interleukin 2, antibacterial and antitumor activity. *EMBO J.* 1995; 14: 1615-1625.

46. Fan W, Shen T, Ding Q, Lv Y, Li L, Huang K, Yan L, Song S. Zearalenone induces ROS-mediated mitochondrial damage in porcine IPEC-J2 cells. *J Biochem Mol Toxicol.* 2017;31: e21944.

47. Grenier B, Bracarense A-PFL, Schwartz HE, Trumel C, Cossalter A-M, Schatzmayr G, Kolf-Clauw M, Moll W-D, Oswald IP. The low intestinal and hepatic toxicity of hydrolyzed fumonisin B1 correlates with its inability to alter the metabolism of sphingolipids. *Biochem. Pharmacol.* 2012; 83: 1465–1473.

48. Kemmerly T, Kaunitz JD. 2014. Gastroduodenal mucosal defense. *Curr Opin Gastroenterol.* 2014; 30: 583-588.

49. Wan LY, Allen KJ, Turner PC, El-Nezami H. Modulation of mucin mRNA (MUC5AC and MUC5B) expression and protein production and secretion in Caco-2/HT29-MTX co-cultures following exposure to individual and combined Fusarium mycotoxins. *Toxicol Sci.* 2014; 139: 83-98.

50. Dupont A, Kaconis Y, Yang I, Albers T, Woltemate S, Heinbockel L, Andersson M, Suerbaum S, Brandenburg K, Hornef MW. Intestinal mucus affinity and biological activity of an orally administered antibacterial and anti-inflammatory peptide. *Gut.* 2015; 64: 222-232.

51. Lien KA, Sauer WC, He JM. Dietary influence on the secretion into and degranulation of mucins in the digestive tract of monogastric and humans. *J Anim Feed Sci.* 2001; 10: 223-245.

52. Petkevicius S, Bach Knudsen KE, Murrell KD. Effects of *Oesophagostomum dentatum* and dietary carbohydrates on morphology of the large intestine of pigs. *Vet Parasitol.* 2003; 116: 125-138.

53. Theodoropoulos G, Hicks SJ, CoWeld AP, Miller BG, Kapel CMO, Trivizaki M, Balaskas C, Petrakos G, Carrington SD. Trichinella spralis: enteric mucin-related reponse to experimental infections in conventional and SPF pigs. *Exp Parasitol.* 2005; 109: 63-71.

54. Huan YW, Bengtsson RJ, MacIntyre N, Guthrie J, Finlayson H, Smith SH, Archibald AL, Ait-Ali T. *Lawsonia intracellularis* exploits β-catenin/Wnt and Notch signalling pathways during infection of intestinal crypt to alter cell homeostasis and promote cell proliferation. *PLoS One*. 2017; 12:e0173782.

55. Gassler N. Paneth cells in intestinal physiology and pathophysiology. *World J Gastrointest Pathophysiol.* 2017; 8:150-160.

56. Kiros TG, Derakhshani H, Pinloche E, D'Inca R, Marshall J, Auclair E, Khafipour E, Van Kessel A. Effect of live yeast Saccharomyces cerevisiae (Actisaf Sc 47) supplementation on the performance and hindgut microbiota composition of weanling pigs. *Sci Rep.* 2018; 8:5315.

57. Che L, Xu Q, Wu C, Luo Y, Huang X, Zhang B, Auclair E, Kiros T, Fang Z, Lin Y, Xu S, Feng B, Li J, Wu. Effects of dietary live yeast supplementation on growth performance, diarrhoea severity, intestinal permeability and immunological parameters of weaned piglets challenged with enterotoxigenic Escherichia coli K88. *Br J Nutr*. 2017; 118:949-958.

58. Oswald IP, Desautels C, Laffitte J, Fournout S, Peres SY, Odin M, Le Bars P, Le Bars J, Fairbrother JM. Mycotoxin fumonisin B-1 increases intestinal colonization by pathogenic *Escherichia coli* in pigs. *Appl Environ Microbiol.* 2003; 69:5870–5874.

59.Verbrugghe E, Vandenbroucke V, Dhaenens M, Shearer N, Goossens J, De Saeger S, Eeckhout M, D'Herde K, Thompson A, Deforce D, Boyen F, Leyman B, Van Parys A, De Backer P, Haesebrouck F, Croubels S, Pasmans F. T-2 toxin induced *Salmonella typhimurium* intoxication results in decreased Salmonella numbers in the cecum contents of pigs, despite marked effects on Salmonella-host cell interactions. *Vet Res*. 2012; 43:22.

60. Chen Y, Chou K, Fuchs E, Havran WL, Boismenu R. Protection of the intestinal mucosa byintraepithelial gamma delta T cells. *Proc Natl Acad Sci USA*. 2002; 99:14338–14343.

61. Hou L, Gan F, Zhou X, Zhou Y, Qian G, Liu Z, Huang K. Immunotoxicity of ochratoxin A and aflatoxin B1 in combination is associated with the nuclear factor kappa B signaling pathway in 3D4/21 cells. *Chemosphere*. 2018; 199: 718-727.

62. Marin DE, Taranu I, Burlacu R, Manda G, Motiu M, Neagoe I, Dragomir C, Stancu M, Calin L. Effects of zearalenone and its derivatives on porcine immune response. *Toxicol In Vitro*. 2011; 25: 1981-1988.

63.Waché YJ, Valat C, Postollec G, Bougeard S, Burel C, Oswald IP, Fravalo P. Impact of deoxynivalenol on the intestinal microflora of pigs. *Int J Mol Sci.* 2009; 10:1-17.

64. Pinton P, Accensi F, Beauchamp E, Cossalter AM, Callu P, Grosjean F, Oswald IP. Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. *Toxicol Lett*. 2008; 177:215-222.

65. Sun H, Pang KS. Permeability, transport, and metabolism of solutes in Caco-2 cell monolayers: a theoretical study. *Drug Met Dispo*. 2007; 36: 102-123.

66. Akbari P, Braber S, Varasteh S, Alizadeh A, Garssen J, Fink-Gremmel S. The intestinal barrier as an emerging target in the toxicologic assessment of mycotoxins. *Arch Toxicol.* 2017; 91:1007–1029.

67. Wan LY, Turner PC, El-Nezami H. Individual and combined cytotoxic effects of *Fusarium* toxins deoxynivalenol, nivalenol, zearalenone and fumonisins B1 on swine jejunal epithelial cells. *Food Chem Toxicol.* 2013; 57:276–283.

68. Alassane‑Kpembi I, Puel O, Pinton P, Cossalter A-M, Chou T-C, Oswald IP. Co‑exposure to low doses of the food contaminants deoxynivalenol and nivalenol has a synergistic inflammatory effect on intestinal explants. *Arch Toxicol.* 2017; 91: 2677-2687.

69. Ling KH, Wan ML, El-Nezami H, Wang M. Protective capacity of resveratrol, a natural polyphenolic compound, against deoxynivalenol- induced intestinal barrier dysfunction and bacterial translocation. *Chem Res Toxicol.* 2016; 29:823–833.

70. Kolf-Clauw M, Castellote J, Joly B, Bourges-Abella N, Raymond-Letron I, Pinton P, Oswald IP. Deelopment of a pig jejunal explant culture for studying the gastrointestinal toxicity of the mycotoxin deoxynivalenol: histopathological analysis. *Toxicol In Vitro.* 2009; 23: 1580-1584.

71. Randall KJ, Turon J, Foster JR. Explant culture of gastrointestinal tissue a review of methods and applications. *Cel Biol Toxicol.* 2011; 27: 267-284.

72. Basso K, Gomes F, Bracarense A. P. Deoxynivanelol and fumonisin, alone or in combination, induce changes on intestinal junction complexes and in E-cadherin expression. *Toxins (Basel).* 2013; 5: 2341-2352.

73. Cheat S, Gerez JR, Cognie J, Alassane-Kpembi I, Bracarense A.P.F.L, Raymond-Letron I, Oswald IP,Kolf-Clauw M. Nivalenol ha a greater impact than deoxynivalenol on pig jejunum mucosa in vitro on explants and in vivo on intestinal loops. *Toxins (Basel).* 2015; 7: 1945-1961.

74. Alshannaq A, Yu J-H. Occurrence, toxicity, and analysis of the major mycotoxins in food. I J *Environ Res Public Health.* 2017; 14: 632.
75. Bouhet S, Oswald IP. The intestine as a possible target for fumonisin toxicity. *Mol Nutr Food Res.* 2007; 51:925–931.

76. Maidana L, Gerez JR, El Khoury R, Pinho F, Puel O, Oswald IP, Bracarense APFRL. Effects of patulin and ascladiol on porcine intestinal mucosa: An ex vivo approach. *Food Chem Toxicol.* 2016; 98: 189-194

77. Saini SS, Kaur A. Aflatoxin B1: toxicity, characteristics and analysis: mini review. *Global Adv Res J Chem Material Sci.* 2012; 1: 63-70.

78. Bennett JW, Klich M, Mycotoxins. Clinical Microbiology Reviews 16: 497–516.
Bhat, P.V., Pandareesh, M.D., Khanum, F., Tamatam, A., 2016. Cytotoxic effects of ochratoxin A in neuro-2a cells: roe of oxidative stress evidenced by N-acetylcysteine. *Front Microbiol.* 2003; 7: 1142 (1-12).

79. Chen X, Naeher K, Applegate TJ. Interactive effects of dietary protein concentration and aflatoxina B1 on performance, nutrient digestibility, and gut health in broiler chicks. *Poultry Sci.* 2016; 95: 1312-1325.

80. Wang F, Zuo Z, Chen K, Gao C, Yang Z, Zhao S, Li J, Song H, Peng X, Fang J, Cui H, Ouyang P, Zhou Y, Shu G, Jing B. Histopathological injuries, ultrastructural changes, and depressed TLR expression in the small intestine of broiler chickens with Aflatoxin $B_1$. *Toxins (Basel).* 2018; 10: pii: E131.

81. Hao S, Hu J, Song S, Huang D, Xu H, Qian G, Gan F, Huang K. Selenium Alleviates Aflatoxin $B_1$-Induced Immune Toxicity through Improving Glutathione Peroxidase 1 and Selenoprotein S Expression in Primary Porcine Splenocytes. *J Agric Food Chem.* 2016; 64 :1385-1393.

82. Liu J, Wang QC, Han J, Xiong B, Sun SC. Aflatoxin B1 is toxic to porcine oocyte maturation. *Mutagenesis.* 2015; 30:527-535.

83. Weaver AC, See MT, Hansen JA, Kim YB, De Souza AL, Middleton TF, Kim SW. The use of feed additives to reduce the effects of aflatoxin and deoxynivalenol on pig growth, organ health and immune status during chronic exposure. *Toxins (Basel).* 2013; 5:1261-81.

84. Lei M, Zhang N, Qi D. In vitro investigation of individual and combined cytotoxic effects of aflatoxin B1 and other selected mycotoxins on the cell line porcine kidney 15. *Exp Toxicol Pathol.* 2013; 65:1149-57.

85. Caloni F, Cortinovis C. Effects of fusariotoxins in the equine species. *Vet J.* 2010; 186: 157-161.

86. Ahangarkani, F, Rouhi, S, Azizi, IG. A review on incidence and toxicity of fumonisins. *Toxin Reviews.* 2014; 33: 95–100.

87. Grenier B, Loureiro-Bracarense AP, Lucioli J, Pacheco GD, Cossalter A-M, Moll W-D, Schatzmayr G, Oswald IP. Individual and combined effects of subclinical doses of deoxynivalenol and fumonisins in piglets. *Mol Nut Food Res.* 2011; 55: 761-771.

88.Voss KA, Smith GW, Haschek WM. Fumonisins: toxicokinetics, mechanism of action and toxicity. *Animal Feed Sci Technol.* 2007; 137: 299-325

89. Loiseau N, Polizzi A, Dupuy A, Therville N, Rakotonirainy M, Loy J, Viadere JL, Cossalter AM, Bailly JD, Puel O, Kolf-Clauw M, Bertrand-Michel J, Levade T, Guillou H, Oswald IP. New insights into the organ-specific adverse effects of fumonisin B1: comparison between lung and liver. *Arch Toxicol.* 2015; 89:1619-1629.

90. Bhat, P.V., Pandareesh, M.D., Khanum, F. and Tamatam, A., 2016. Cytotoxic effects of ochratoxin A in neuro-2a cells: role of oxidative stress evidenced by N-acetylcysteine. *Front Microbiol.* 2016; 7: 1142.

91. Malir F, Ostry V, Pfohl-Leszkowicz A, Malir J, Toman J. Ohratoxin A: 50 years of research. *Toxins.* 2016; 8: 191.

92. Gayathri L, Dhivya R, Dhanasekaran D, Periasamy VS, Alshatwi A A, Akbarsha MA. Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: *in vitro* study in HepG2 cell. *Food Chem Toxicol.* 2015; 83: 151-163.

93. Aydin G, Ozçelik N, Ciçek E, Soyöz M. Histopathologic changes in liver and renal tissues induced by Ochratoxin A and melatonin in rats. *Human Exp Toxicol.* 2003; 22: 383-391.

94. Tanaka T, Hasegwa-Baba Y, Watanabe Y, Mizukami S, Kangawa Y, Yoshida T, Shibutani M. Maternal exposure to ochratoxin A targets intermediate progenitor cells of hipocampal neurogenesis in rat offspring via cholinergic signal downregulation and oxidative stress reponses. *Reprod Toxicol.* 2016; 65: 113-122.

95. Costa JG, Saraiva N, Guerreiro PS, Louro H, Silva MJ, Miranda JP, Castro M, Batinic-Haberle I, Fernandes AS, Oliveira NG. Ochratoxin A-induced cytotoxicity, genotoxicity and reactive oxygen species in kidney cells: an integrative approach of complementary endpoints. *Food Chem Toxicol.* 2016; 87: 65-76.

96. Ramya P, Padma W. Ochratoxin-induced toxicity, oxidative stress and apoptosis ameliorated by quercetin-modulation of Nrf2. *Food Chem Toxicol.* 2013; 62: 205-216.

97. Qian G, Lu D, Hu J, Gan F, Hou L, Chen X, Huang K. Ochraoxin A-induced autophagy *in vitro* and *in vivo* promotes porcine circovirus type 2 replication. *Cell Death Dis.* 2017; 8: e2909.

98. Mally A, Dekant W. Mycotoxins and the kidney: Modes of action for renal tumor formation by ochratoxin A in rodents. *Mol Nutr Food Res.* 2009; 53: 467–478.

99. Zhou Y, Gan F, Hou L, Zhou X, Adam Ibrahim YA, Huang K. Modulations of DNMT1 and HDAC1 are involved in the OTA-induced cytotoxicity and apoptosis in vitro. *Chem Biol Interact.* 2017; 25: 170-178.

100. Zhang Z, Gan F, Xue H, Liu Y, Huang D, Khan AZ, Chen X, Huang K. Nephropathy and hepatopathy in weaned piglets provoked by natural ochratoxin A and involved mechanisms. *Exp Toxicol Pathol.* 2016; 68: 205-13.

101. Marin DE, Pistol GC, Gras MA, Palade ML, Taranu I. Comparative effect of ochratoxin A on inflammation and oxidative stress parameters in gut and kidney of piglets. *Regul Toxicol Pharmacol.* 2017; 89:224-231.

102. Romero A, Ares I, Ramos E, Castellano V, Martinez M, Martinez-Larranaga MR, Anadon A, Martinez MA. 2016. Mycotoxins modify the barrier function of Caco-2 cells through differential gene expression of specific claudin isoforms: protective effect of illite mineral clay. *Toxicol.* 2016; 353-354: 21-33.

103. Ranaldi G, Mancini E, Ferruzza S, Sambuy Y, Perozzi G. Effects of red wine on ochratoxin A toxicity in intestinal Caco-2/TC7 cells. *Toxicol in Vitro.* 2007; 21: 204-210.

104. Maresca M, Yahi N, Younes-Sakr L, Boyron M, Caporiccio B, Fantini J. Both direct and indirect effects account for the pro-inflammatory activity of enteropathogenic mycotoxins on

the human intestinal epithelium: stimulation of interleukin-8 secretion, potentiation of interleukin-1 beta effect and increase in the transepithelial passage of commensal bacteria. *Toxicol Appl Phamacol.* 2008; 228: 84-92.

105. Tannous J, Keller NP, Atoui A, Elkhoury A, Lteif R, Oswald IP, Puel O. Secondary metabolism in Penicillium expansum: emphasis on recent advances in patulin research. *Crit Rev Food Sci Nutr.* 2017; 31: 1-17.

106. Assuncao R, Alvito P, Kleiveland CR, Lea TE. Characterization of in vitro effects of patulin on intestinal epithelial and immune cells. *Toxicol Lett.* 2016; 250-251: 47-56.

107. Jayashree GV, Krupashree K, Rachitha P, Khanum F. Patulin induced oxidative stress mediated apoptotic damage in mice, and its modulation by Green Tea Leaves. *J Clin Exp Hepatol.* 2017; 7: 127-134.

108. Boussabbeh, M, Ben Salem, I., Belquesmi, F., Neffati, F., Najjar, M.F., Abi-Essefi S, Bacha, H. Crocin protects the iver and kidney from patulin-induced apoptosis *in vivo*. *Environ Sci Poll Res Int.* 2016; 23: 9799-9808.

109. Lu X, Zhang E, Yin S, Fan L, Hu H. Methylselenic acid prevents patulin-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress and inactivation of p53 and MAPKs. *J Agric Food Chem*. 2017; 65: 5299-5305.

110. Jin H, Yin S, Song X, Zhang E, Fan L, Hu H. P53 activation contributes to patulin-induced nephrotoxicity via modulaton of reactive oxygen species generation. *Sci Rep*. 2016; 6: 24455.

111. McLaughlin J, Lambert D, Padfield PJ, Burt JP, O'Neil CA. The mycotoxin patulin, modulates tight junctions in Caco-2 cells. . *Toxicol In Vitro*. 2009; 23: 83-89.

112. Katsuyama A, Komo T, Shimoyama S, Kikuchi H. The mycotoxins patulin decreases expression of density-enhanced phosphatase-1 by down-regulating PPARgamma in human colon cancer cells. *Tohoku J Exp Med.* 2014; 223: 265-274.

113. Keblys M, Bernhoft A, Hofer CC, Morrison E, Larsen HJ, Flaoyen A. The effects of the Penicillium mycotoxins citrinin, cyclopiazonic acid, ochratoxin A, patulin, penicillic acid, and roquefortine C on in vitro proliferation of porcine lymphocytes. *Mycopathol.* 2004; 158: 317-24.

114. Heussner AH, Dietrich DR, O'Brien E. In vitro investigation of individual and combined cytotoxic effects of ochratoxin A and other selected mycotoxins on renal cells. *Toxicol In Vitro*. 2006; 20: 332-41.

115. Zwierzchowski W, Obremski K, Zielonka Ł, Skorska-Wyszyńska E, Gajecka M, Jakimiuk E, Polak M, Jana B, Rybarczyk L, Gajecki M. The impact of zearalenone on the level of the selected strogens in blood serum of sexually immature gilts. *Pol J Vet Sci.* 2006; 9: 247-252.

116. Koraichi F, Videmann B, Mazallon M, Benahmed M, Prouillac C, Lecoeur S. Zearalenone exposure modulates the expression of ABC transporters and nuclear receptors in pregnant rats and fetal liver. *Toxicol Lett.* 2012; 1: 246–256.

117. Parveen M, Zhu Y, Kiyama R. Expression profiling of the genes responding to zearalenone and its analogues using estrogen-responsive genes. *FEBS Letters*. 2009; 583: 2377-2384.

118. Frizzell C, Ndossi D, Verhaegen S, Dahl E, Eriksen G, Sorlie M, Ropstad E, Muller M, Elliott CT, Connolly L. Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis. *Toxicol Lett*. 2011; 206: 210-217.

119. Tiemann U, Danicke S. *In vivo* and *in vitro* effects of the mycotoxins zearalenone and deoxynivalenol on different non-reproductive and reproductive organs in female pigs: a review. *Food Add Contam*. 2007; 24: 306–314.

120. Reddy KE, Lee W, Jeong JY, Lee Y, Lee HJ, Kim MS, Kim DW, Yu D, Cho A, Oh YK, Lee SD. Effects of deoxynivalenol- and zearalenone-contaminated feed on the gene expression profiles in the kidneys of piglets. *Asian-Australas J Anim Sci.* 2018; 31: 138-148.

121. Pestka JJ. Deoxynivalenol mechanism of action, human exposure, and toxicological relevance. *Arch Toxicol.* 2010; 84: 663-679.

122. Payros D, Alassane-Kpembi I, Pierron A, Loiseau N, Pinton P, Oswald IP. Toxicology of deoxynivalenol and its acetylated and modified forms. *Arch Toxicol.* 2016; 90: 2931-2957.

123. Pinton P, Braicu C, Nougayrede JP, Laffitte J, Taranu I, Oswald IP. Deoxynivalenol impairs porcine intestinal barrier function and decreases the protein expression of claudin-4 through a mitogen-activated protein kinase-dependent mechanism. *J Nutr.* 2010; 140:1956–1962.

124. Dąbrowski M, Obremski K, Gajęcka M, Gajęcki MT, Zielonka Ł. Changes in the Subpopulations of Porcine Peripheral Blood Lymphocytes Induced by Exposure to Low Doses of Zearalenone (ZEN) and Deoxynivalenol (DON). *Molecules.* 2016; 21: pii: E557

125. Gerez JR., Desto SS, Bracarense APFRL. Deoxynivalenol induces toxic effects in the ovaries of pigs: An ex vivo approach. *Theriogenol.* 2017; 90: 94-100.

126. Broekaert N, Devreese M, Demeyere K, Berthiller F, Michlmayr H, Varga E, Adam G, Meyer E, Croubels S. Comparative *in vitro* cytotoxicity of modified deoxynivalenol on porcine intestinal epithelial cells. *Food Chem Toxicol.* 2016; 95: 103-109.

127. Park SH, Kim J, Kim D, Moon Y. Mycotoxin detoxifiers attenuate deoxynivalenol-induced proinflammatory barrier insult in porcine enterocytes as an in vitro evaluation model of feed mycotoxin reduction. *Toxicol In Vitro*. 2017; 38:108-116.

128. Cheat S, Pinton P, Cossalter AM, Cognie J, Vilarino M, Callu P, Raymond-Letron I, Oswald IP, Kof-Clauw M. The mycotoxins deoxynivalenol and nivalenol show in vivo synergism on jejunum enterocytes apoptosis. *Food Chem Toxicol.* 2016; 87: 45-54.

129. Del Regno M, Adesso S, Popolo A, Quaroni A, Autore G, Severino L, Marzocco S. Nivalenol induces oxidatve stress and increases deoxynivalenol pro-oxidant effect in intestinal epitelial cells. *Toxicol Appl Pharmacol.* 2015; 285: 118-127.

130. Luongo D, De Luna R, Russo R, Severino L. Effects of four Fusarium toxins (fumonisin B(1), alpha-zearalenol, nivalenol and deoxynivalenol) on porcine whole-blood cellular proliferation. *Toxicon.* 2018; 98: 698-706.

131. Alassane‐Kpembi I, Puel O, Oswald IP. Toxicological interactions between the mycotoxins deoxynivalenol, nivalenol and their acetylated derivatives in intestinal epithelial cells. *Arch Toxicol.* 2005; 89: 1337-1346.

132. Lattanzio VM, Visconti A, Haidukowski M, Pascale M. Identification and characterization of nem Fusarium masked myscotoxins, T2 and HT2 glycosyl derivatives, in naturally contaminated whet and ots by liquid chromatography-high-resolution mass spectrometry. *J Mass Spectrom*. 2012; 47: 466-475.

133. Quiroga MA, Risso MA, Perfumo CJ. T-2 mycotoxin intoxication in piglets: a systematic pathological approach and apoptotic immunohistochemical studies. *Braz J Vet Pathol*. 2009; 2: 16-32.

134. Agrawal M, Yadav P, Lomash V, Bhaskar AS, Lakshmana Rao PV. T-2 toxin induced skin inflammation and cutaneous injury in mice. *Toxicol.* 2012; 302: 255-265.

135. Antonissen G, Martel A, Pasmans F, Ducatelle R, Verbrugghe E, Vanderbroucke VLS, Haesebrouck F, Van Immerseel F, Croubels S. The impact of Fusarium mycotoxins on humans and animal host susceptibility to infectious diseases. *Toxins (Basel).* 2014; 28: 430-452.

136. Adhikari M, Negi B, Kaushik N, Adhikari A, Al-Khedhairy AA, Kaushik NK, Choi E. T-2 mycotoxin: toxicological effects and decontamination strategies. *Oncotarget*. 2017; 8: 33933-33952.

137. Seeboth J, Solinhac R, Oswald IP, Guzylack-Piriou L. The fungal T-2 toxin alters the activation of primary macrophages induced by TLR-agonists resulting in a decrease of the inflammatory response in the pig. *Vet Res.* 2012; 24:35.

138. Horvatovich K, Hafner D, Bodnár Z, Berta G, Hancz C, Dutton M, Kovács M. Dose-related genotoxic effect of T-2 toxin measured by comet assay using peripheral blood mononuclear cells of healthy pigs. *Acta Vet Hung*. 2013; 61: 175-186.

139. Goossens J, De Bock L, Osselaere A, Verbrugghe E, Devreese M, Boussery K, Van Bocxlaer J, De Backer P, Croubels S. The mycotoxin T-2 inhibits hepatic cytochrome P4503A activity in pigs. *Food Chem Toxicol.* 2013; 57: 54-56.

140. Capcarova M, Petruska P, Zbynovska K, Kolesarova A, Sirotkin AV. Changes in antioxidant status of porcine ovarian granulosa cells after quercetin and T-2 toxin treatment. *J Environ Sci Health B*. 2015; 50: 201-206.

141. Goossens J, Pasmans F, Verbrugghe E, Vandenbroucke V, De Baere S, Meyer E, Haesebrouck F, De Backer P, Croubels S. Porcine intestinal epithelial barrier disruption by the Fusarium mycotoxins deoxynivalenol and T-2 toxin promotes transepithelial passage of doxycycline and paromomycin. *BMC Vet Res.* 2012; 8:245.

142. Obremski K, Podlasz P, Zmigrodzka M, Winnicka A, Woźny M, Brzuzan P, Jakimiuk E, Wojtacha P, Gajecka M, Zielonka Ł, Gajecki M. The effect of T-2 toxin on percentages of CD4+, CD8+, CD4+ CD8+ and CD21+ lymphocytes, and mRNA expression levels of selected cytokines in porcine ileal Peyer's patches. *Pol J Vet Sci.* 2013; 16:341-349.

143. Del Río García JC, Moreno Ramos C, Pinton P, Mendoza Elvira S, Oswald IP. 2007. Evaluation of the cytotoxicity of aflatoxin and fumonisin in swine intestinal cells. *Rev Iberoam Micol.* 2007; 24: 136-141.

144. Tulayakul P, Dong KS, Li JY, Manabe N, Kumagai S. The effect of feeding piglets with the diet containing green tea extracts or coumarin on in vitro metabolism of aflatoxin B1 by their tissues. *Toxicon.* 2007; 50: 339-348.

145. Bouhet S, Hourcade E, Loiseau N, Fikry A, Martinez S, Roselli M, Galtier P, Mengheri E, Oswald IP. The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. *Toxicol Sci.* 2004; 77:165–171.

146. Bouhet S, Le Dorze E, Peres S, Fairbrother JM, Oswald IP. Mycotoxin fumonisin B1 selectively down-regulates the basal IL-8 expression in pig intestine: *In vivo* and *in vitro* studies. *Food Chem. Toxicol.* 2006; 44:1768–1773.

147. Loiseau N, Debrauwer L, Sambou T, Bouhet S, Miller JD, Martin PG, Viadere JL, Pinton P, Puel O, Pineau T, Tulliez J, Galtier P, Oswald IP. Fumonisin B1 exposure and its selective effect on porcine jejunal segment: sphingolipids, glycolipids and trans-epithelial passage disturbance. *Biochem Pharmacol.* 2007; 74:144–152.

148. Marin DE, Motiu M, Taranu I. Food contaminant zearalenone and its metabolites affect cytokine synthesis and intestinal epithelial integrity of porcine cells. *Toxins (Basel).* 2015; 7:1979–1988.

149. Braicu C, Selicean S, Cojocneanu-Petric R, Lajos R, Balacescu O, Taranu I, Marin DE, Motiu M, Jurj A, Achimas-Cadariu P, Berindan-Neagoe I. Evaluation of cellular and molecular impact of zearalenone and Escherichia coli co-exposure on IPEC-1 cells using microarray technology. BMC Genomics. 2016; 17: 576.

150. Fan W, Lv Y, Ren S, Shao M, Shen T, Huang K, Zhou J, Yan L, Song S. Zearalenone (ZEA)-induced intestinal inflammation is mediated by the NLRP3 inflammasome. Chemosphere. 2018; 190:272-279.

151. Obremski K, Gajecka M, Zielonka L, Jakimiuk E, Gajecki M. Morphology and ultrastructure of small intestine mucosa in gilts with zearalenone mycotoxicosis. *Pol J Vet Sci.* 2005; 8: 301-307.

152. Obremski K, Zielonka L, Gajecka M, Jakimiuk E, Bakuła T, Baranowski M, Gajecki M. Histological estimation of the small intestine wall after administration of feed containing deoxynivalenol, T-2 toxin and zearalenone in the pig. *Pol J Vet Sci.* 2008; 1:339-345.

153. Obremski K. The effect of in vivo exposure to zearalenone on cytokine secretion by Th1 and Th2 lymphocytes in porcine Peyer's patches after in vitro stimulation with LPS. *Pol J Vet Sci.* 2014; 17: 625-632.

154. Przybylska-Gornowicz B, Tarasiuk M, Lewczuk B, Prusik M, Ziółkowska N, Zielonka Ł, Gajecki M, Gajecka M. The effects of low doses of two Fusarium toxins, zearalenone and deoxynivalenol, on the pig jejunum. A light and electron microscopic study. *Toxins (Basel).* 2015; 7: 4684–4705.

155. Lewczuk B, Przybylska-Gornowicz B, Gajecka M, Targonska K, Ziółkowska N, Prusik M, Gajecki M. Histological structure of duodenum in gilts receiving low doses of zearalenone and deoxynivalenol in feed. *Exp Toxicol Pathol.* 2016; 68: 157–166.

156. Obremski K. Changes in Th1 and Th2 cytokine concentrations in ileal Peyer's patches in gilts exposed to zearalenone. *Pol J Vet Sci.* 2014; 17: 53-59.

157. Obremski K, Gonkowski S, Wojtacha P. Zearalenone-induced changes in the lymphoid tissue and mucosal nerve fibers in the porcine ileum. Pol J Vet Sci. 2015; 18: 357-365.

158. Obremski K, Wojtacha P, Podlasz P, Żmigrodzka M. The influence of experimental administration of low zearalenone doses on the expression of Th1 and Th2 cytokines and on selected subpopulations of lymphocytes in intestinal lymph nodes. *Pol J Vet Sci.* 2015; 18: 489-497.

159. Obremski K, Poniatowska-Broniek G. Zearalenone induces apoptosis and inhibits proliferation in porcine ileal Peyer's patch lymphocytes. Pol J Vet Sci. 2015; 18: 153-161.

160. Joa H, Konga C, Song M, Kim BG. Effects of dietary deoxynivalenol and zearalenone on apparent ileal digestibility of amino acids in growing pigs. *Anim. Feed Sci. Technol.* 2016; 219: 77–82.

161. Diesing AK, Nossol C, Panther P, Walk N, Post A, Kluess J, Kreutzmann P, Danicke S, Rothkotter HJ, Kahlert S. Mycotoxin deoxynivalenol (DON) mediates biphasic cellular response in intestinal porcine epithelial cell lines IPEC-1 and IPEC-J2. *Toxicol Lett.* 2011; 200:8–18.

162. Vandenbroucke V, Croubels S, Martel A, Verbrugghe E, Goossens J, Van Deun K, Boyen F, Thompson A, Shearer N, De Backer P, Haesebrouck F, Pasmans F. The mycotoxin deoxynivalenol potentiates intestinal inflammation by Salmonella typhimurium in porcine ileal loops. *PLoS ONE*. 2011; 6:e23871.

163. Gu MJ, Song SK, Park SM, Lee IK, Yun CH. Bacillus subtilis protects porcine intestinal barrier from deoxynivalenol via improved zonula occludens-1 expression. *Asian-Australas J Anim Sci.* 2014; 27:580–586.

164. Lucioli J, Pinton P, Callu P, Laffitte J, Grosjean F, Kolf-Clauw M, Oswald IP, Bracarense AP. The food contaminant deoxynivalenol activates the mitogen activated protein kinases in the intestine: interest of ex vivo models as an alternative to in vivo experiments. *Toxicon.* 2013; 66:31-6.

165. Pinton P, Graziani F, Pujol A, Nicoletti C, Paris O, Ernouf P, Di Pasquale E, Perrier J, Oswald IP, Maresca M. Deoxynivalenol inhibits the expression by goblet cells of intestinal mucins through a PKR and MAP kinase dependent repression of the resistin-like molecule β. *Mol Nutr Food Res.* 2015; 59:1076-1087

166. Alizadeh A, Braber S, Akbari P, Garssen J, Fink-Gremmels J. Deoxynivalenol impairs weight gain and affects markers of gut health after low-dose, short-term exposure of growing pigs. *Toxins (Basel).* 2015; 7:2071–2095.

167. Lessard M, Savard C, Deschene K, Lauzon K, Pinilla VA, Gagnon CA, Lapointe J, Guay F, Chorfi Y. Impact of deoxynivalenol (DON) contaminated feed on intestinal integrity and immune response in swine. *Food Food Chem Toxicol.* 2015; 80:7–16.

168. Hedman R, Thuvander A, Gadhasson I, Reverter M, Pettersson H. Influence of dietary nivalenol exposure on gross pathology and selected immunological parameters in young pigs. *Nat Toxins.* 1997; 5:238-46.

169. Goyarts T, Grove N, Dänicke S. Effects of the Fusarium toxin deoxynivalenol from naturally contaminated wheat given subchronically or as one single dose on the in vivo protein synthesis of peripheral blood lymphocytes and plasma proteins in the pig. *Food Chem Toxicol.* 2006; 44:1953-65.

170. Kolf-Clauw M, Sassahara M, Lucioli J, Rubira-Gerez J, Alassane-Kpembi I, Lyazhri F, Borin C, Oswald IP. The emerging mycotoxin, enniatin B1, down-modulates the gastrointestinal toxicity of T-2 toxin in vitro on intestinal epithelial cells and ex vivo on intestinal explants. *Arch Toxicol.* 2013; 87:2233-2241.

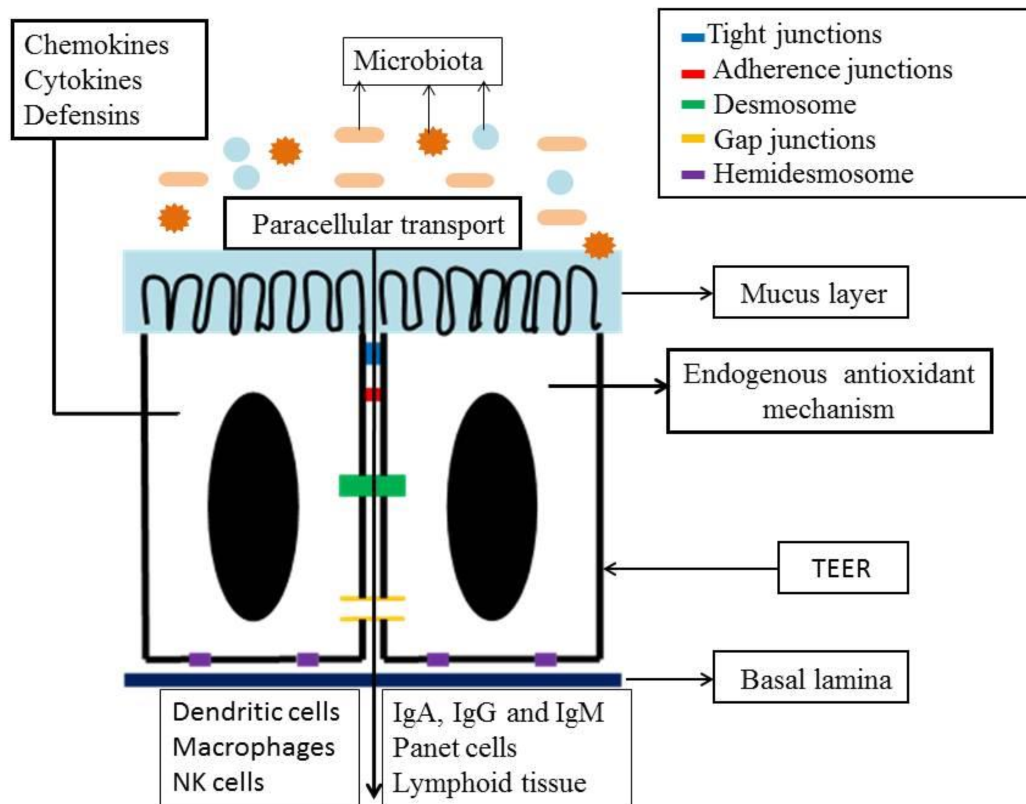



**Figure 1**. Components of maintenance, integrity, and defense associated with the IEB.

**Table 1**.Toxic effects of mycotoxins AFB1, FB1, OTA, PAT and ZEN on the IEB compounds of pigs.

| Mycotoxin | Model | Dose | Duration | Results | Reference |
|---|---|---|---|---|---|
| **AFB1** | ***In vitro*:** | | | | |
| | IPEC-1 cells | 1.3 -100 µM | 48 h | -Increase of IL-8 (1.3 and 2 µM)<br>-Decrease of IL-8 (50 and 100 µM) | Del Rio Garcia et al [143] |
| | ***In vivo*:** | | | | |
| | Piglets | 200,000 dpm | 10-60 m | -Increase of DNA adduct formation<br>- Decrease of GSH and GST | Tulayakul et al [144] |
| **FB1** | ***In vitro*** | | | | |
| | IPEC-1 cells | 50–500 µM | 16-28 h | -Decrease of TEER<br>-Decrease of IL-8 | Bouhet et al [145]<br>Bouhet et al [146]<br>Loiseau et al [147] |
| | IPEC-2J cells | 2.5-40µM | 48 h | -Decrease of cell viability<br>-Increase of IL-1α, IL-1β, IL-6, IL-8, TNF-α and MCP-1. | Wan et al [67]<br>Wan et al [43] |
| | | 20–200 µM | 4 h | -Increase in translocation of pathogenic *Escherichia coli* | Bouhet and Oswald [20] |
| | ***Ex vivo* :** | | | | |
| | Jejunal explants | 10 -70µM | 2-3 h | -Increase in TEER<br>-Increase in permeability of HRP<br>-Villi atrophy and fusion<br>-Vacuolation and flattening of enterocytes<br>-Increase of caspase-3 expression | Lalles et al [24]<br>Silva et al [23] |
| | ***In vivo*:** | | | | |
| | Piglets (proximal and distal small intestine) | 2.8 µM/Kg bw | 14 days | -Decrease of villi height<br>-Villi atrophy and fusion<br>-Lymphatic vessel dilation<br>-Interstitial edema<br>-Deccrease of IL-1β, Il-10, IL-2 and IFN-γ | Grenier et al [47] |
| | Piglets (small and large intestine) | 0.5 mg/ kg bw | 7 days | -Increase translocation of pathogenic *Escherichia coli*<br>-Downregulation of IL-8 | Oswald et al [58]<br>Bouhet et al [146] |
| | Piglets (jejunum and Ileum) | 6 mg/kg feed | 28 days | Villi atrophy and fusion<br>-cytoplasmic vacuolation of enterocytes<br>-apical necrosis of villi<br>-edema of lamina propria<br>-Decrease of cell proliferation<br>-Increase of lymphocytes, eosinophils and plasma cells in lamina propria<br>-Upregulation of IL-10 and IFN-γ (jejunum)<br>-Upregulation of TNF-α and IL-1β (ileum)<br>-Decrease the OCL and E-cadherin expression | Bracarense et al [7] |
| **OTA** | ***In vivo*:** | | | | |
| | Piglets (duodenum and colon) | 0.05 mg/kg feed | 30 days | -Downregulation of NF-κB, iNOS, IL-6, IL-8, IL-12, IL-17 expression.<br>-Decrease the Nrf2 gene expression.<br>-Increase SOD. | Marin et al [101] |
| **PAT** | ***Ex vivo*:** | | | | |
| | jejunal explants | 10-100 µM | 4h | -Decrease of goblet cells.<br>-Increase of cell apoptosis. | Maidana et al [76] |
| **ZEN** | ***In vitro*:** | | | | |
| | IPEC-1 cells | 10-50 µM | 24 h | -Decrease the cell viability.<br>-Downregulation of IL-6, IL-8 and TNF-α | Marin et al [148]<br>Braicu et al [149] |
| | IPEC-J2 cells | 6 -50µg/mL | 48 h | -Increase MDA<br>-Decrease GSH, CAT, SOD and MMP<br>-Decrease of cell viability.<br>-Up-regulation of pBD-1 and 2 expression<br>-Upregulation of NLRP3 inflmmasome, pro-IL-1β, pro-IL-18 and caspase I | Wan et al [67]<br>Fan et al [46]<br>Fan et al [150] |
| | | 2.5-40 µM | 48 h | -Up-regulation of pBD-1 and 2 expression<br>-Decrease of cell viability | Wan et al [67]<br>Wan et al [43] |
| | PBMC cells | 5-10 µM | 7 days | -Upregulation of IgA, IgG and IgM | Marin et al [62] |

**Table 1.**Toxic effects of mycotoxinns AFB1, FB1, OTA, PAT and ZEN on the IEB compounds of pigs (continuation).

| Mycotoxin | Model | Dose | Duration | Results | Reference |
|---|---|---|---|---|---|
| | ***In vivo:*** | | | | |
| **ZEN** | Gilts (small intestine) | 200-400 µg/kg bw | 7 days | -Increase the Paneth cells on the bottom of intestinal crypts<br>-Increased activity of the goblet cells | Obremski et al[151] |
| | Pigs | 33.2 µg/kg feed | 14 days | -Decrease of goblet cells<br>-Increase of inflammatory cells (lymphocytes and plasma cells) | Obremski et al[152] |
| | Gilts (jejunum, duodenum) | 8 µg /Kg bw | 42 days | -Upregulation of IL-4 and IL-10 | Obremsky [153] |
| | | 40 µg /Kg bw | 42 days | -Increase the goblet cells (villi)<br>- Increase the lymphocytes into mucosal epithelium and lamina propria<br>-Changes in microvilli distribution | Przybylska-Gornowicz et al[154]<br>Lewczuk et al [155] |
| | Gilts/pigs (Ileal Peyer's patches) | 100 µg/kg feed | 42 days | -Increase lymphocyte apoptosis<br>-Upregulation of IL-12 and IL-1β<br>-Decrease of $CD21^+$ expression on B cells<br>-Decrease the NK cells proliferation | Obremski[156]<br>Obremski et al[157]<br>Obremski et al[158]<br>Obremski and Poniatowska-Broniek[159] |
| | Growing pigs (ileum) | 10 mg /kg bw | 3 days | -Affect digestibility of tryptophan amino acid | Joa et al [160] |
| | Piglets (jejunum and ileum) | 1.5 mg/kg feed | 28 days | -Decrease of villi height and goblet cells.<br>-Atropy and fusion villi, and edema of lamina propria | Gerez et al[13] |
| | Sows/growing pigs (jejunum) | 2.77 mg /kg bw | 35/70 days | -Decrease of villi height<br>- Decrease SOD<br>-Increase MDA and GPx<br>-Upregulation of IL-1β, IL-6 and TNF-α<br>-Changes in microflora | Liu et al[36] |

AFB1- Aflatoxin B1; FB1-Fumonisin B1; OTA-Ocratoxin A; PAT-Patulin; ZEN-Zearalenone
Bw: body weight; CAT-catalase; GSH- gluthatione; GST-glutathione S-transferase; GPx- glutathione peroxidase; HRP-horseradish peroxidase; MCP-1- monocyte chemoatractant protein-1; MDA- malondialdehyde; MMP- mitochondrial membrane permeability; OCL-ocludin; PBMC- peripeheral blood mononuclear cells; SOD-

**Table 2.**Toxic effects of trichothecenes (DON, NIV and T2-toxin) on the IEB compounds of pigs.

| Mycotoxin | Model | Dose | Duration | Results | References |
|---|---|---|---|---|---|
| **DON** | ***In vitro***: | | | | |
| | IPEC-1 cells | 0.67-150 µM | 24-48 h | -Decrease the cell viability.<br>-Decrease the TEER<br>-Decrease the CLD4 and ZO-1 expression.<br>-Increase in permeability to 4 kDa FITC-dextran.<br>- Increase in translocation of commensal E*scherichia coli*.<br>-Increase the caspase 3 expression. | Pinton et al[21]<br>Pinton et al[123]<br>Diesing et al[161]<br>Alassane-Kpembi et al[12] |
| | IPEC-J2 cells | 0.67-33.7 µM | 12-72 h | -Decrease the CLDN3, CLDN4 and ZO-1 expression.<br>-Decrease cell viability.<br>-Increase in permeability to 4 kDa FITC-dextran.<br>- Increase in translocation of pathogenic *Salmonella Typhimurium*.<br>- Increase in translocation of E*scherichia coli*<br>-Increase the caspase-3 expression.<br>-Increase DNA fragmentation.<br>-Increase the MAPKs p38, ERK and JNK activation.<br>-Increase the Il-12, IL-6, IL-8 and TNF-α expression<br>-Up-regulation of pBD-1 and 2 expression | Diesing et al[161]<br>Vandenbroucke et al[162]<br>Goossens et al[141]<br>Wan et al[43]<br>Gu et al[163]<br>Ling et al[69] |
| | ***Ex vivo*:** | | | | |
| | jejunal explants | 1-10µM | 2-4 h | - Increase permeability to 4 kDa FITC-dextran<br>-Villi atrophy and fusion<br>-Loss of apical enterocytes, - Apical villi necrosis<br>-Changes in enterocytes morphology.<br>-Decrease mucin production<br>-Increase the caspase-3 and Cox-2 expression.<br>-Decrease the E-cadherin expression. | Pinton et al[21]<br>Basso et al[72]<br>Lucioli et al[164]<br>Silva et al[23]<br>Cheat et al[73]<br>Pinton et al[165] |
| | ***In vivo:*** | | | | |
| | Pigs (jejunum) | 28.9 µg/kg/feed | 14 days | -Decrease goblet cells number<br>-Increase of lymphocytes and plasma cells (lamina propria) | Obremski et al[152] |
| | Piglets (duodenum,jejunum, ileum and colon) | 0.9-3.5 mg/Kg feed | 10 -28days | -Villi atrophy and fusion<br>-Edema of lamina propria<br>-Apical villi necrosis.<br>-Decrease in villi height and crypt depth.<br>-Decrease of goblet cells –- Decrease of lymphocytes and plasma cells in lamina propria.<br>-Decrease epithelial cell proliferation<br>-Decrease of E-cad, CLDN3, CLDN4, ZO-1 and 2 expression.<br>-Increase IL-1β, IL-2, IL-6, IL-12p40, MIP-1β and TNF-α expression.<br>-Changes in the vaccinal immunoglobulin expression<br>-Changes in the microbiota. | Pinton et al[64]<br>Pinton et al[21]<br>Waché et al[63]<br>Bracarense et al[7]<br>Alizadeh et al[166]<br>Cheat et al[73]<br>Gerez et al[13]<br>Lessard et al[167] |

**Table 2.** Toxic effects of trichothecenes (DON, NIV and T2-toxin) on the IEB compounds of pigs (continuation).

| Mycotoxin | Model | Dose | Duration | Results | References |
|---|---|---|---|---|---|
| **DON** | ***In vivo:*** | | | | |
| | Piglets (jejunum loops) | 3.5 mg/kg feed +1-10 µM | 28 days+24 h | -Increase epithelial cells apoptosis | Cheat et al[128] |
| | Piglets (jejunum and ileum) | 1.3- 2.5 mg/kg feed | 3-4 weeks | -Atropy and fusion villi<br>-Edema of lamina propria<br>-Flattening of enterocytes<br>-Loss of apical enterocytes.<br>-Decrease of villi height and goblet cells<br>-Decrease epithelial cell proliferation<br>-Increase epithelial and immune cells apoptosis<br>-Increase IgA | Hedman et al[168]<br>Cheat et al[73]<br>Gerez et al[13] |
| | Pigs (serum blood) | 5.7 mg/kg feed | 4 weeks | -Increase IgG and IgM | Goyarts et al[169] |
| | Piglets (serum blood) | 2.2-2.5mg/kg feed | 9 weeks | -Increase IgA | Pinton et al[64] |
| | Gilts (Jejunum, duodenum) | 12 µg/ kg bw | 6 weeks | -Increase the mucosal thickness.<br>-Increase the crypt depth<br>-Increase the goblets cells number (villi).<br>- Increase the lymphocytes into mucosal epithelium and lamina propria | Przybylska-Gornowicz et al[154]<br>Lewczuk et al[155] |
| **NIV** | ***In vitro:*** | | | | |
| | IPEC-1 cells | 0.12-150 µM | 24 h | -Decrease the cell viability | Alassane-Kpembi et al[131] |
| | IPEC-J2 cells | 0.25-2 µM | 48 h | -Up-regulation of pBD-1 and 2 expression.<br>-Decrease the cell viability. | Wan et al[67]<br>Wan et al[43] |
| | ***Ex vivo*:** | | | | |
| | jejunal explants | 1,3 and 10µM | 4 h | -Villi atrophy and fusion<br>-Apical denudation of villi<br>-Edema in lamina propria<br>-Flattening of enterocytes | Cheat et al[73] |
| | ***In vivo:*** | | | | |
| | Piglets (jejunum and ileum) | 1.3- 2.5 mg/kg feed | 3-4 weeks | -Atropy and fusion villi<br>-Edema of lamina propria<br>-Flattening of enterocytes<br>-Loss of apical enterocytes.<br>-Decrease of villi height and goblet cells<br>-Decrease epithelial cell proliferation<br>-Increase epithelial and immune cells apoptosis<br>-Increase IgA | Hedman et al[168]<br>Cheat et al[73]<br>Gerez et al[13] |

**Table 2.**Toxic effects of trichothecenes (DON, NIV and T2-toxin) on the IEB compounds of pigs (continuation).

| Mycotoxin | Model | Dose | Duration | Results | References |
|---|---|---|---|---|---|
| **T2-toxin** | ***In vitro*:** IPEC-1 cells | 0.3-100 nM | 48 h | -Decrease the cell proliferation -Increase the villi fusion -Loss of apical enterocytes -Edema in lamina propria | Kolf-Clauw et al[170] |
| | IPEC-2J cells | 0.21-210 nM | 1-72 h | -Decrease the cell viability -Decrease the TEER Increase in permeability of doxycycline and paromomycin -Increase in translocation of *Salmonella typhimurium* | Goossens et al[141] Verbrugghe et al[59] |
| | ***Ex vivo*:** jejunal explants | 0.3-100 nM | 4h | -Increase the villi fusion -Loss of apical enterocytes -Edema in lamina propria | Kolf-Clauw et al[170] |
| | ***In vivo*:** Piglets (duodenum, jejunum, ileum, cecum and colon) | 1.5-2.5 mg/ kg bw | 16-35 h | -Submucosal edema -Necrosis of epithelial cells in crypt region -Depletion and necrosis of ileal Peyer's patches -Increase of apoptosis | Quiroga et al[133] |
| | Pigs (jejunum) | 11.5 µg/kg feed | 14 days | -Decrease goblet cells -Increase lymphocytes and plasma cells (lamina propria) | Obremski et al[152] |
| | Piglets (small intestine) | 15-83 µg/kg feed | 23 days | -Increase the troaslocation of *Salmonella typhimurium* | Verbrugghe et al[59] |
| | Piglets (Ileal Payer's patches) | 200 µg/kg feed | 14-42 days | -Decrease the IL-4 and IL-10 expression -Decrease the $CD21^{+}$ expression on B cells | Obremski et al[142] |

AFB1- Aflatoxin B1; FB1-Fumonisin B1; OTA-Ocratoxin A; PAT-Patulin; ZEN-Zearalenone
bw: body weight; CAT-catalase; CLDN3-claudin 3; CLDN4- Claudin 4; Cox-2: cyclooxygenase-2; GSH- gluthatione; GST - glutathione S-transferase; GPx- glutathione peroxidase; HRP-horseradish peroxidase; MCP-1- monocyte chemoatractant protein-1; MDA- malondialdehyde; MMP- mitochondrial membrane permeability; PBMC- peripeheral blood mononuclear cells; SOD- superoxide dismutase; TEER- transepithelial electrical resistance; ZO-1- zonula occluden 1; ZO-2- zonula occluden 2.

## 2.2 EFFECTS OF PHYTIC ACID ON INTESTINE: *IN VITRO*, *EX VIVO* AND *IN VIVO* STUDIES

### Introduction

Phytic acid (IP6) is found mainly in vegetables and grains and its protective effect has been related to its antioxidant potential in inhibiting radical oxygen species (ROS) production (GRAF; EATON, 1985; VUCENIK; SHAMSUDDIN, 2006). However, little is known about the other possible interactions and signals of this acid in human and animal cells. This review describes the properties and biological fucntions of IP6, and summarize its protective effects on intestine in several pathological conditions.

### Chemical, biological properties and intracellular functions

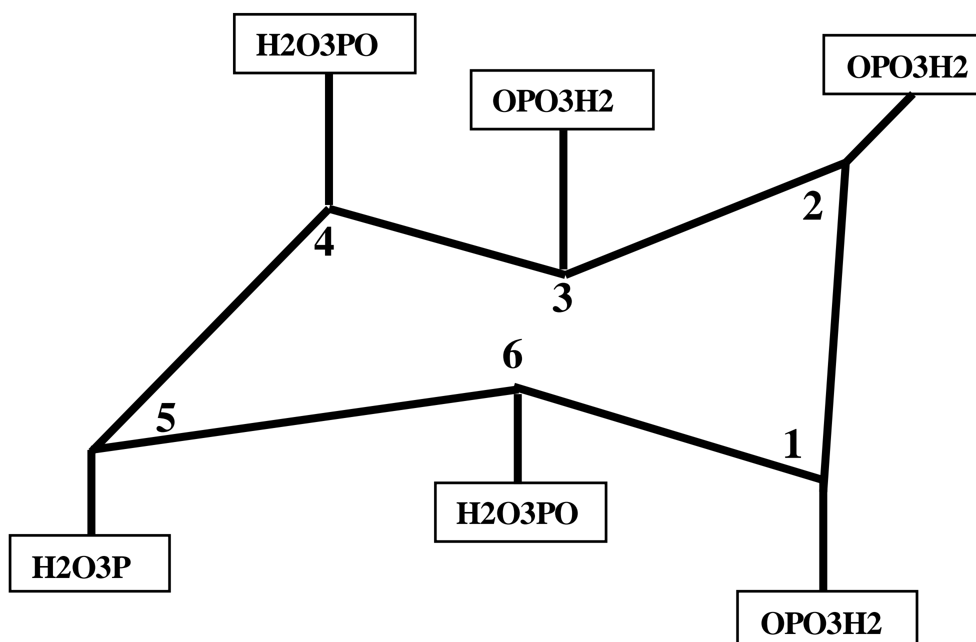
Phytic acid (IP6, InsP6, inositol hexaphosphate, inositol hexaphosphate or myo-inositol-1 2,3,4,5,6 - hexaphosphate) is a natural antioxidant found naturally in the form of salts ( phytate Na2Mg5, K2Mg5 and Ca2Mg5) in cereals, vegetables, nuts and natural oils (SILVA et al., 2016), and comprises 60-90% of the total phosphorus present in the seeds used in food and feed (LOLAS et al., 1976; GRAF; EATON, 1990). The grouping and position of phosphates at 1,2 and 3 (axial-equatorial-axial) is responsible for specific interaction with iron (Figure 1). This biological interaction results in highly stable complexes and inhibits the Fenton reaction, which is associated with their antioxidant capacity (GRAF; EATON, 1990).

IP6 has been considered as an anti-nutritional factor due the high affinity for polyvalent cations such as Cu2+, Zn2, Ni2+, Co2+, Mn2+, Fe3+ and Ca2 (GRAF; EATON, 1990). However, the chelation with minerals is dependent on several factors, including the proportion of phytate: metal and the pH in the gastrointestinal tract (GRAF; EATON 1990). Moreover, in balanced diets, the inhibitory effects of IP6 in the binding of minerals is low (SCHLEMMER et al., 2009).

Studies in human and animal models have shown the preventive and therapeutic effects of IP6 on the inhibition of platelet aggregation (VUCENIK et al., 1999), inhibit metabolic disturbances (ONOMI et al., 2004; FOSTER et al., 2017), neurodegenerative (Anekonda et al., 2011) and cardiovascular (OBATA; NAKASHIMA, 2016) diseases. In addition, IP6 has been associated with the prevention of kidney stone formation and

osteoporosis (FUSTER et al., 2017), and presented anti-inflammatory and anticancer effects (VUCENIK et al., 2005; KAPRAL et al., 2015).

**Figure 1.** Molecular structure and configuration of IP6.




In mammalian cells, the inositol is mainly found in cell membranes as phosphatidylinositol. The inositol polyphosphates has an important role in the control of several cellular functions, such as endocytosis (ZI et al., 2000), exocytosis (EFANOV et al., 1997), traffic of ions and proteins traffic (SHEARS, 1996), export of mRNA from the nucleus to the cytoplasm (YORK et al., 1999), DNA repair (MA; LIEBER, 2002), protein enveloping (MACBETH et al., 2005), cell division and differentiation (MENNITI et al., 1993), and oocyte maturation (JI et al., 1989). Moreover, the inositol phosphates present complex patterns of interaction in the phosphorylation and dephosphorylation cycles, renewing the *pools* of intracellular inositol phosphate (SEEDS et al., 2005).

## Intestinal effect of IP6

At the intestinal level, the IP6 is rapidly uptaken by cells, transported to the intracellular region by pinocytosis and/or endocytosis receptor-mediated, and dephosphorylated into inositol phosphates with fewer phosphate groups (VUCENIK;

SHAMSUDDIN, 1994). This rapid dephosphorylating of IP6 may be associated with beneficial effects on the growth, differentiation and cell cycle regulation (VUCENIK; SHAMSUDDIN, 2006).

Most studies have used rats and mice as animal models to study the intestinal effects of IP6 (Table 1). Nonetheless, pigs have similar characteristics with humans regarding the degradation and absorption of IP6, which occurs mostly in the stomach, upper portion of the small intestine and colon (SCHLEMMER et al., 2001). This similarity makes pigs the most suitable experimental model for the study of the effects of IP6 on cancer, inflammatory diseases, and other diseases, especially in comparison with laboratory animals. The intestinal favorable effects of IP6 are dose-dependent and were demonstrated mainly by modulation of morphological changes, cell proliferation and apoptosis, transport of proteins and inflammatory response as summarized in Table 1.

*Modulalation of morphological changes*

The aberrant crypt foci (ACF) are groups of abnormal tube-like glands in the mucosa of the colon and rectum. ACF is a precursor change before colorectal polyps formation and is one of the earliest tissue alteration seen in the colon that may result in cancer development (OCHIAI et al., 2014). Rodents are the main experimental model to study chemical toxicity and diets that might prevent the ACF and colorectal cancer (ALMAGRAMI et al., 2014; ROUHOLLAHI et al., 2015; REHMAN et al., 2018). The administration of IP6 in drinking water (PRETLOW et al., 1992) and feed (CHALLA et al., 1997) reduced the incidence of ACF induced by azoxymethane in the colon of rats. In pigs, IP6 reduced the histological changes such as atrophy and villi fusion, epithelial degeneration and necrosis, flattening of enterocytes and increased the villi height in jejunal explants exposed to fumonisin B1 (FB1) and deoxynivalenol (DON), (SILVA et al., 2014). The modulation of these intestinal morphological changes are associated with IP6 capacity to induce reversal of abnormal cells back to well-differentiated phenotype, modulate the cell proliferative stages, and inhibit the oxidative stress (YANG; SHAMSUDDIN, 1995; SILVA et al., 2014; LIU et al., 2015).

**Table 1**- Protective effects of IP6 on intestinal tissue in pathological conditions.

| Pathological Condition | Model | IP6 dose | Duration | Results | Reference |
|---|---|---|---|---|---|
| Colon cancer | *In vitro:* | | | | |
| | Caco-2 | 1-5 mM | 1-24 h | -Decrease of iNOS expression.<br>-Decrease of TGF-β1<br>-Decrease of IL-8 expression<br>-Decrease of TNF-α expression<br>-Increase of TGF-β2 and TGF-β 3<br>-Inhibition of p38 MAPK | CHOLEWA et al., 2008;<br>WAWSZCZYK et al., 2012;<br>KAPRAL et al., 2013;<br>KAPRAL et al., 2015;<br>KAPRAL et al., 2017. |
| | | 1- 10 mM | 12-24 h | -Decrease of cell proliferation<br>-Decrease of cells in S phase (maintenance in G1-phase)<br>-Decrease of AKT1 and S6K1<br>-Increase of p21 and CDNK1 B<br>-Increase caspase 9 e 3 | |
| | HT-29 cells | 100-400 µg/mL | 12-48 h | -Decrease cell proliferation<br>-Decrease PI3K and AKT of expression<br>-Decreas of ephosphorylation of AKT (pAKT)<br>-Increase of apoptosis<br>-Arrest of Go/G1 phase cell | NURUL-HUSNA et al., 2010;<br>LIU et al., 2015 |
| | | 9.5-14.5µg/mL | 24-72 h | -Decrease of cell proliferation<br>-Increase of Bax expression<br>-Decrease of Bcl-xl expression<br>-Increase of caspase-8 and 3 expression | SHAFIE et al., 2013. |
| | | 0.2- 5 mM | 24-72 h | -Decrease of the cell proliferation<br>-Increase of caspase-3 activation | SCHROTEROVA et al., 2010. |
| | | 0.33-20 mM | 1-6 d | -Decrease of DNA synthesis<br>-Decrease of PCNA expression<br>-Decrease of mucin marker GalGaINAC<br>-Increase of alkaline phosphatase activity<br>-Induction of cell differentiation | YANG; SHAMSUDDIN, 1995. |
| | *In vivo:* | | | | |
| | *Mice* | 80 mg/bw | 20 d | -Decrease tumoral growth<br>-Decrease of EMC proteins collagen IV, LN and FN in tumoral metastasis microenvironment<br>-Decrease of integrin-β1, MMP-9, VEGF and TGF-β expression in tumor | FU et al., 2016 |
| | Rats | 0.2-0.5% w/v (drinking water) | 8-20 w | -Decrease of β-catenin expression<br>-Decrease of ki-67 expression<br>-Decrease of Cox-2 expression<br>-Decrease of incidence of ACF | LATIFAH et al., 2010;<br>NORAZALINA et al, 2013;<br>SAAD et al., 2013 |
| | | 1-2% (drinking water) | 13-20 w | -Decrease of incidence of ACF<br>-Increased of natural killer cells activity | CHALLA et a., 1997;<br>ZHANG et al., 2005. |
| | | 0.25-1 g/kg bw (gavage) | 39 w | -Inhibited tumor growth<br>-Decrease of AKT expression<br>-Decrease of c-Myc<br>-Increase of inactive β-catenin | YU et al., 2017. |
| High fat diet | *In vivo*: | | | | |
| | Rats | 1.02% feed | 21 d | -Increase of *Lactobacillus* spp.<br>-Increase of mucin production<br>-Increase of β-glucoronidase activity<br>-Decrease of TNF-α and IL-6 | SEKITA et al., 2016. |
| Mycotoxins exposure | *In vitro*: | | | | |
| | IPEC-1 | 0.5-1 mM | 24 h | -Increase of the cell viability<br>-Increase of TEER | PACHECO et al., 2012. |
| | *Ex vivo*: | | | | |
| | Jejunal explants | 2.5 – 5 mM | 4 h | -Decrease of morphological changes<br>-Decrease of ki-67 expression<br>-Decrease of caspase 3 expression<br>-Decrease of cox-2 expression | SILVA et al., 2014. |

ACF- aberrant crypt foci; TEER- transepithelial electrical resistance

*Modulation of cell proliferation and apoptosis*

IP6 could regulate the cell cycle to block uncontrolled cell division and force malignant cell either to differentiation by binding to the insulin-like growth factor II (IGF-II), decreasing DNA synthesis, proliferating cell nuclear antigen (PCNA), ki-67 protein and blocking ERK 1/2 mitogen-activated protein kinase (MAPK) signaling to arrest the cells in

the G0/G1 phase of the cell proliferation cycle (SHAMSUDDIN, 1999; SILVA et al., 2014; SILVA et al., 2016).

Several studies have demonstrated that IP6 increase the apoptosis in intestinal tumoral cells (SCHROTEROVÁ et al., 2010; SHAFIE et al., 2013; LIU et al., 2015). Alternatively, our group have demonstrated that IP6 can protect the intestinal cells against mycotoxins-induced apoptosis in intestinal explants of swine (SILVA et al., 2014) similar to inhibition of apoptosis in rat dopaminergic neural cell line (N-27) exposed to iron (XU et al., 2006). These results suggest that IP6 modulates apoptosis to protect the cells and to prevent the development of pathological changes.

IP6 can induce cell apoptosis through the up-regulation of the Bax mitochondrial (intrinsic pathway) (SHAFIE et al., 2013). The up-regulation of Bax by IP6 induce cytochrome C release from the mitochondria in combination with caspase-9, apoptosis-activating factor 1 (Apaf-1) and adenosine triphosphate (ATP) to process procaspase-3 which then activates caspase-3 (KATUNUMA et al., 2001; LI et al., 2004).

In addition, the role of IP6 on apoptosis modulation can be associated to antioxidant effects and modulation of inositol hexaphosphato kinase-2 (Insp6K2) action, since the deletion of this enzyme prevents apoptosis and the translocation of Insp6K2 from nuclei to mitochondria is associated with cell death (NAGATA et al., 2016).

*Antioxidant effects and modulation of immunological response*

Studies have demonstrated that IP6 can modulate the inflammatory response of IEC through regulating the expression and secretion of cytokines, and chemokines (KAPRAL et al., 2013). IP6 decreases the expression of pro-inflammatory cytokines and chemokines that play role in the maintenance of inflammatory response. This anti-inflammatory action of IP6 was observed in inflammatory bowel diseases studies (CHOLEWA et al., 2008; KAPRAL et al., 2013).

Due their high antioxidant capacity, the IP6 inhibit lipid peroxidation and subsequent mechanisms associated to inflammatory response such as mitogen-activated protein kinases (MAPks) activation, nitric oxide synthase (iNOS) and ciclooxigenase-2 (Cox-2) expression. The oxidative stress can induce the activation of p38 MAPK that stimulate the proinflammatory cytokines expression such TNF-α, IL-1, IL-6 and IL-8 (LEE et al,1994). In addition, the activation of p38 MAPK was associated to expression of Cox-2 (CUENDA; ROUSSEAU, 2007). In addition, the IP6 decrease the expression of Cox-2 by inhibition of

rate-limiting step to catalize the conversion of arachidonic acid into prostaglandins and suppression of β-catenin activity (SAAD et al., 2013).

The nitric oxide synthase (iNOS) family catalyzes the synthesis of nitric oxide (NO) and it can be regulated at the transcriptional level in response to proinflammatory cytokines (DAVIES et al., 1995). The activation of iNOS induce to prolonged overproduction of NO with cytotoxic effects by increase of membrane lipid peroxidation and permeability, DNA damage and suppression of DNA repair enzymes (LYONS, 1995). At intestinal level the overproduction of NO was associated to chronic inflammatory response (KAPRAL et al., 2015). The IP6 demonstrated important action as inhibitor of the iNOS transcription in Caco-2 cells stimulate with pro-inflammatory agents (KAPRAL et al., 2015).

*Effects of IP6 on P-glycoprotein efflux*

The P-glycoprotein (P-gp) is a member of the ATP-binding cassette (ABC) transporter superfamily (UEDA et al., 2011). In the IEC, the P-gp is located mainly in the apical membrane and mediates the efflux of fargoing substrates, protect the tissue against toxic effects of xenobiotics and regulates drugs absorption and intracellular disposition (UEDA et al., 2011; SILVA et al., 2015). P-gp substrates such as antiviral (HIV protease inhibitor), antipsychotic (perospirone), antiarrhythmic (digoxin) and anticancer (vincristine) can specifically bind with P-gp and be transported out of the cell by this molecule, causing insufficient intracellular drug concentrations and decreasing of therapeutic efficacy (SILVA et al., 2015). The IP6 inhibits P-gp efflux and is thereby a potential P-gp inhibitor, and can co-administrated with p-gp substrate drugs to enhance their therapeutic effects (LI et al., 2018).

**Conclusion**

Studies have demonstrated benefic effects of IP6 on intestine in inflammatory and carcinogenic diseases, and mycotoxins exposition through antioxidant and anti-inflammatoy action, and modulation of apoptosis and cell proliferation. The intracellular pathways and interactions of IP6 in the intestine are not completely understood, and more studies are necessary to elucidate its effects, preventive and therapheutic applications.

**References**

ALMAGRAMI, A.A.; ALSHAWSH, M.A.; SAIF-ALI, R.; SHWTER, A.; SALEM, S.D.; ABDULLA, M.A. Evaluation of chemopreventive effects of Acanthus ilicifolius against azoxymethane-induced aberrant Crypt Foci in the rat colon. *PLoS One*, v.12, n.5, e96004, 2014.

ANEKONDA, T.S. WADSWORTH, T.L.; SABIN,R.; FRAHLER, K.; HARRIS,C.; PETRIKO, B.; RALLE, M.; WOLTJER, R.; QUINN, J.F. Phytic acid as a potential treatment for Alzheimer's pathology: evidence from animal and in vitro models. *Journal of Alzheimer's Disease,* v .23, n.1, p. 21-35, 2011.

BERRIDGE, M.J.; IRVINE, R.F. Inositol phosphates and cell signaling. *Nature*, v. 341, n. 6239, p. 197-205, 1989.

CHALLA, A.; RAO, D.R.; REDDY, B.S. Interactive suppression of aberrant crypt foci induced by azoxymethane in rat colon by phytic acid and green tea. *Carcinogenesis,* v.18, n.10, p. 2012-2016, 1997.

CHOLEWA, K.; PARFINIEWICA, B.; BEDNAREK, I.; SWIATKOWSKA, L. JEZIENICKA, E.; KIEROT,J.; WEGLARZ, L. The influence of phytc acid on TNF-α and its receptors genes expressin in colon cancer Caco-2 cells. *Acta Poloniae Pharmaceutica-Drug Research*, v. 65, n.1, p. 75-79, 2008.

CUENDA, A.; ROUSSEAU, S. p38 MAP-Kinases pathway regulation, function and role in human diseases. *Biochimica et Biophysica Acta* , v.1773, . 1358-1375, 2007.

DAVIES, M.G.; FULTON, G.J.; HAGEN, P.O. Clinical biology of nitric oxide. *British Journal of Surgery*, v. 82, p. 1598-1610, 1995.

EFANOV, A.M.; ZAITSEV, S.V. BERGGREN, P.O. Inositol hexakisphosphate stimulates non-$Ca^{2+}$-mediated and primers $Ca^{2+}$-mediated exocytosis of insulin by activation of protein kinase C. *Proceedings of National Academy of Sciences*, v. 94, p. 4435-4439, 1997.

FOSTER, S.R.; DILWORTH, L.L.; THOMPSON, R.K.; ALEXANDER-LINDO, R.L.; OMORUYI, F.O. Effects of combined inositol hexakisphosphate and inositol supplement on antioxidant activity and metabolic enzymes in the liver of streptozotocin-induced type 2 diabetic rats. C*hemico-Biolgical Interactions,* v. 275, p. 108-115, 2017.

FU, M.; SONG, Y.; WEN, Z.; LU, X.; CUI, L. Inositol hexaphosphate and inositol inhibit colorectal cancer metastasis to the liver in Balb/c mice. *Nutrients*, v. 8, p. 286, 2016.

FUSTER, J.M.B.; CORTES, S.P.; BESTARD, J.P.; FREIXEDAS, F. Plant phosphates, phytate and pathological calcifications in chronic kidney disease. *Nefrologia*, v. 37, n. 1, p. 20-28, 2017.

GRAF, E.; EATON, J.W. Dietary suppression of colonic cancer. Fiber or phytate? *Cancer*, v.56, n.4 p. 717-718, 1985.

GRAF, E.; EATON, J.W. Antioxidant functions of phytic acid. *Free Radical Biology & Medicine*, v. 8, n.1, p. 61-69, 1990.

JI, H.; SANDBERG, K.; BAUKAL, A.J.; CATT, K.J. Metabolism of inositol pentakisphosphate to inositol hexakisphosphate in *xenopus laevis* oocytes. T*he Journal of Biological Chemistry*, V. 264, , N.34, P. 20185-20188, 1989.

KAPRAL, M.;WAWSZCZYK, J.; JESSE, K.; PAUL-SAMOJEDNY, M.; KUSMIERZ, D.; LUDMIŁA WEGLARZ, L. Inositol hexaphosphate inhibits proliferation and induces apoptosis of colon cancer cells by suppressing the AKT/mTOR signaling pathway. *Molecules*, v. 22, n. 10, p.1657, 2017.

KAPRAL, M.; WAWSZCYK, J.; SMOLIK, S.; WEGLARZ, L. Transcriptional regulation of interleukin 6 and its receptor in colon cancer cells by phytic acid. *Acta Poloniae Pharmaceutica-Drug Research*, v. 67, n.6, 701-705, 2010.

KAPRAL, M.; WAWSZCZYK, J.; SOSNICKI, S.; WEGLARZ, L. Down-regulation of inducible nitric oxide synthase expression by inositol hexaphosphate in human colon cancer cells. *Acta Poloniae Pharmaceutica-Drug Research*, v. 72, n. 4, p. 705-711, 2015.

KAPRAL, M.; WAWSZCZYK, J.; SOSNICKI, S.; WEGLARZ, L. Differential influence of inositol hexaphosphato on the expression of genes encoding TGF-β isoforms and their receptors in intestinal epithelial cells stimulated with proinflammatory agents. *Mediators of Inflammation*, v. 2013, ID 436894, 2013.

KATUNUMA, N.; MATSUI, A.; LE, Q.T.; UTSUMI, K.; SALVESEN, G.; OHASHI, A. Novel procaspase-3 activating cascade mediated by lysoapoptases and its biological significances in apoptosis. *Advances in Enzyme Regulation,* v**.**41, p. 237–250, 2001.

LATIFAH, S.Y.; ARMANIA, N.; TZE, T.H.; AZHAR, Y.; NORDIANA, A.H.; NORAZALINA, S.; HAIRUSZAH, I.; SAIDI, M.; MAZNAH, I. Germinated brown rice (gbr) reduces the incidence of aberrant crypt foci with the involvement of beta-catenin and cox-2 in azoxymethane-induced colon cancer in rats. *Nutrition Journal,* v. 26, n.9, p. 16, 2010.

LEE, J.C.; LAYDON, J.T.; McDONNELL, P.C.; GALLAGHER, T.F.; KUMAR,S.; GREEN, D.; McNULTY, D.; BLUMENTHAL, M.J.; HEYS, J.R.; LANDYATTER, S.W.; STRICKLER, J.E.; MCLAUGHLIN, M.M.; SIEMENS, I.R.; FISHER, S.M.; LIVI, G.P.; WHITE, J.R.; ADAMS, J.L.; YOUNG, P.R. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, *Nature,*v. 372, n. 6508, p.739-1994.

LI, L.;, FU, Q.; XIA, M.; XIN, L.; SHEN, H.; LI, G.; JI, G.; MENG, Q.; XIE, Y. Inhibition of P-glycoprotein mediated efflux in Caco-2 cells by phytic acid. *Journal of Agricultural and Food Chemistry*, v. 66, n.4, p. 988-998, 2018.

LI, P.; NIJHAWAN, D.; WANG, X. Mitochondrial activation of apoptosis. *Cell*, v.116 (Suppl 2), p. S57-S59, 2004.

LIU, G.; SONG,Y.; CUI, L.; WEN, Z.; LU, X. Inositol hexaphosphate suppresses growth and induces apoptosis in HT-29 colorectal cancer cells in culture: PI3K/Akt pathway as a potential target. *International Journal of Clinical and Experimental Pathology*, v.8, n. 2, p. 1402-1410, 2015.

LOLAS, G.M.; PALAMIDIS, N.; MARKASIS, P. The Phytic acid-total phosphorus relation in barley, oats, soybeans and wheat. *Cereal Chemistry Journal*, v. 53, p. 867-871, 1976.

LYONS, C.R. The role of nitric oxide in inflamation. *Advances in Immunology*, v. 60, p.323-355, 1995.

MA, Y, LIEBER, MR. Binding of inositol hexaphosphato (IP6) to ku but no to DNA-pkcs. *The Journal of Biological Chemistry*, v. 277, n. 13, p. 10756-10759, 2002.

MACBETH, M.R.; SCHUBERT, H.L.; VANDERMARK, A.P.; LINGAM, A.T.; HILL, C.P. BASS, B.L. Inositol hexakisphosphate is bound to adar2 core and require for RNA eding. *Science*, v. 309, n. 5740, p. 1534-1539, 2005.

MENNITI, F.S.; OLIVER, K.G.; PUTNEY, J.W. JR.; SHEARS, SB. Inositol hexaphosphato and cell signaling: new review of insp5 and insp6. *Trends in Biochemical Science*, v. 18, n.2, p. 53-56, 1993.

NAGATA, E.; NONAKA, T.; MORIYA, Y.; FUJII, N.; OKADA, Y.; TSUKAMOTO, H.; ITOH, J.; OKADA, C.; SATOH, T.; ARAI, T.; HASEGAWA, M.; TAKIZAWA, S. Inositol hexakisphosphate kinase 2 promotes cell death in cells with cytoplasmic tdp-43 aggregation. molecular neurobiology. Molecular Neurobiology, v. 53, n.8, p. 5377-5383, 2016.

NORAZALINA, S.; NORHAIZAN, M.E.; HAIRUSZAH, I.; NORASHAREENA, M.S. Anticarcinogenic efficacy of Phytic acid extracted from rice bran on azoxymetahne-induced colon carcinogenensis in rats. *Experimental and Toxicologic Pathology*, v. 62, n. 3, p. 259-268, 2010.

NURUL-HUSNA, S.; NORHAIZAN, M.E.; HAIRUSZAH, I.; NORAZALINA, A.S.; NRSHARINA, I. Rice bran phytic acid (IP6) induces growth inhibition, cell cycle arrest and apoptosis on human colorectal adenocarcinoma cells. *Journal of Medicinal Plants Research*, v.4, n. 21, p. 2283-2289, 2010.

OBATA, T.; NAKASHIMA, M. Phytic acid suppresses ischemia-induced hydroxyl radical generation in rat myocardium. *European Journal of Pharmacology*, v.774, p. 20-24, 2016.

OCHIAI, M.; HIPPO, Y.; IZUMIYA, M.; WATANABE, M.; NAKAGAMA, H. Newly defined aberrant crypt foci as a marker for dysplasia in the rat colon. *Cancer Science*, v. 105, n.8, p. 943-950, 2014.

ONOMI, S.; OKAZAK, Y.; KATAYAMA, T. Effect of dietary level of phytic acid on heptic and serum lipid status in rats fed a high-sucrose diet. *Bioscience, Biotecnology & Biochemistry*, v. 68, n.6,p. 1379-1381, 2004.

PACHECO, G.D.; SILVA, C.A.; PINTON, P.; OSWALD, I.P.; BRACARENSE, A.P. Phytic acid protects porcine intestinal epitelial cells from deoxynivalenol (DON) cytotoxicity. *Experimental and Toxicologic Pathology*, v.64, n.4, p. 345-347, 2012.

PRETLOW, T.P.; O'RIORDAN, M.A. SOMICH, G.A.; AMINI, S.B.; PRETLOW, T.G. Aberrant crypts correlate with tumor incidence in F344 rats treated with azoxymethane and phytate. *Carcinogenesis*, v.13, n. 9, p. 1509-1512, 1992.

REHMAN, M.U.; RAHMAN, M. M.U.; FAROOQ, A., RASHID, S.M.; AHMAD, B.; BILAL AHMAD, S.; ALI, R.;, HUSSAIN, I.; MASOODI, M.; MUZAMIL, S.; MADKHALI, H.; AHMAD GANAIE, M. Naringenin (4,5,7-trihydroxyflavanone) suppresses the development of precancerous lesions via controlling hyperproliferation and inflammation in the colon of Wistar rats. *Environmental Toxicology*, v.33, n.4, p. 422-435, 2018.

ROUHOLLAHI, E.; MOGHADAMTOUSI, S.Z.; AL-HENHENA, N.; KUNASEGARAN, T.; HASANPOURGHADI, M.; LOOI, C.Y.; ABD MALEK, S.N.; AWANG, K.; ABDULLA, M.A.; MOHAMED, Z. The chemopreventive potential of Curcuma purpurascens rhizome in reducing azoxymethane-induced aberrant crypt foci in rats. *Drug Design, Development and Therapy*, v. 27, n.9, p. 3911-3922, 2015.

SAAD, N.; ESA, N.M.; ITHNIN, H. Suppression of β-catenin and cyclooxygenase-2 expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice phytic acid (PA). Asian Pacific Journal of Cancer Prevention, v.14, n.5, p. 3093-3099, 2013.

SCHLEMMER, U.; JANY, K.D.; BERK, A.; SCHULZ, E.; RECHKEMMER, G. Degradation of phytate in the gut of pigs-pathway of gastrointestinal inositol phosphate hydrolysis and enzyme involved. *Archives of Animal Nutrition*, v.55, n. 4, p. 255-280, 2001.

SCHLEMMER, U.; FROLICH, W.; PRIETO, R.M.; GRASES, F. Phytate on foods and significance for humans: food source, intake, processing, bioavailability role and analysis. *Molecular Nutrition and Food Research*, v. 53, Suppl,.2, p. S330-S375, 2009.

SCHEROTEROVA, L.; HAKOVA, P.; RUDOLF, E.; CERVINKA, M. Effect of phytic acid and inositol on the proliferation and apoptosis of cells derived from colorectal carcinoma. *Oncology Reports*, v. 23, n. 3, p. 787-793, 2010.

SEEDS, A.M.; BASTIDAS, R.J.; YORK, D.J. Molecular definition of novel inositol polyphosphate metabolic pathway initiated by inositol 1,4,5-triphosphate 3-kinase activity in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, v. 280, n. 30, p. 27654-27661, 2005.

SEKITA, A.; OKAZAKI, Y.; KATAYAMA, T. Dietary phytic acid prevents fatty liver by reducing expression of hepatic lipogenic enzymes and modulates gut microflora in rats fed a high sucrose-diet. *Nutrition*, v. 32, n.6, p. 720-722, 2016.

SHAFIE, N.H.; ESA, N.M.; ITHNIN, H.; SAAD, N.; PANDURANGAN, A.K. Pro-Apoptotic Effect of Rice Bran Inositol Hexaphosphate (IP6) on HT-29 Colorectal Cancer Cells. *International Journal of Molecular Sciences*, v. 14, n. 12, p. 23545-23558, 2013.

SHAMSUDDIN, A.M. Metabolism and cellular functions of IP6: a review. *Anticancer Research*, v. 19, n. 5A, p. 3733-3736, 1999.

SHEARS, S.B. Inositol pentakis- and hexakisphosphate metabolism adds versatility to the actions of inositol polyphosphates. Novel effects on ion channels and protein traffic. *Subcellular Biochemistry*, v 26, p. 187-226, 1996.

SILVA, E.O.; BRACARENSE, A.P.F.R.L. Phytic acid: from antinutritional to multiple protection factor of organic systems. *Journal of Food Science*, v. 81, n.6, R1357, 2016.

SILVA, E.O.; GEREZ, J.R.; DRAPE, T.C.; BRACARENSE, A.P.F.R.L. Phytic acid decreases deoxynivalenol and fumonisin B1-induced changes on swine jejunal explants. *Toxicology Reports*, v. 1, p. 284-292, 2014.

SILVA, R.; VILAS-BOAS, V.; CARMO, H.; DINIS-OLIVEIRA, R. J.; CARVALHO, F.; DE LOURDES BASTOS, M.; REMIAO, F. Modulation of P-glycoprotein efflux pump: induction and activation as a therapeutic strategy. *Pharmacology & Therapheutics*, v.149, p.1-123, 2015.

UEDA, K. ABC proteins protect the human body and maintain optimal health. *Bioscience, Biotechnology Biochemistry,* v. 75, n. 3, p.401-409, 2011.

VUCENIK, J.; PODEZASY, J.J.; SHAMSSUDDIN, A.M. Antiplatelet activity of inositol hexaphosphate (IP6). *Anticancer Research*, v. 19, n. 5A, p. 3689-3693, 1999.

VUCENIK, I.; SHAMSUDDIN, A.M. Protection against cancer by dietary IP6 and inositol. *Nutrition and Cancer*, v. 55, p. 109-125, 2006.

VUCENIK, I.; SHAMSUDDIN, A.M. Inositol hexaphosphate (phytic acid) is rapidlly absorbed and metabolized by murine an human malignant cells in vitro. *Journal of Nutrition*, v. 124, n. 6, p. 861-868, 1994.

VUCENIK, I.; TANTIVEJKUL, K.; RAMAKRISHNA, G.; ANDERSON, L.M.; RAMLJAK, D. Inositol hexaphosphato (IP6) block proliferation of breast cancer cells through pkcδ-dependent increase in p27kip 1 and decrease in retinoblastoma protein (pRb) phosphorylation. *Breast Cancer Research Treatment*, v. 91, n. 35-45, 2005.

XU, Q.; KANTHASAMY, G.; REDDY, M.B. Phytic acid protects against 6-OHDA and iron induced apoptosis in cell culture model of Parkinson's disease. *FASEB Journal*, v. 20, A192, 2006.

YANG, G-Y.; SHAMSUDDIN, A.M. IP6-induced growth inhibition and differentiation of HT-29 human colon cancer cells: involvement of intracellular inositol phosphate. Anticancer Research, v.15, n.6B, p. 1479-1488, 1995.

YORK, J.D.; ODOM, A.R.; MURPHY, R.; IVES, E.B.; WENTE, S.R. A phospholipase c-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science*, v. 285, n. 5424, p. 96-100, 1999.

YU, W.; LIUB, C.; LIC, X.; YANGB, F.; CHENGA, L.; LIUA, C.; SONGA, Y. Inositol hexaphosphate suppresses colorectal cancer cell proliferation via the Akt/GSK-3β/β-catenin signaling cascade in a 1,2-dimethylhydrazine induced rat model. *European Journal of Pharmacology*, v. 805, p. 67-74, 2017.

ZHANG, A.; SONG, Y. WANG, X.L. Inositol hexaphosphato-induced enhancement of natural killer cell activity correlates with suppression of colon carcinogenesis in rats. *World Journal of Gastroenterology*, v. 11, n. 32, p. 5044-5046, 2005.

ZI, X.; SINGH, R.P.; AGARWAL, R. Impairment of erbB1 receptor and fluid phase endocytosis and associated mitogenic signaling by inositol hexaphosphato in human prostate carcinoma DU145 cells. *Carcinogenesis*, v. 21, n. 12, p. 225-2235, 2000.

Wageningen Academic Publishers

World Mycotoxin Journal, 2018; 11 (1): 113-133
**SPECIAL ISSUE**: *10 years World Mycotoxin Journal*

# 2.3 Mycotoxins and oxidative stress: where are we?

**E.O. da Silva[1], A.P.F.L. Bracarense[1*] and I.P. Oswald[2*]**

*[1]Universidade Estadual de Londrina, Laboratory of Animal Pathology, Campus Universitário, Rodovia Celso Garcia Cid,*
*Km 380, Londrina, Paraná 86051-990, Brazil; [2]Université de Toulouse, Toxalim, Research Center in Food Toxicology,*
*INRA, UMR 1331 ENVT, INP-PURPAN, 31076 Toulouse, France; ana.bracarense@pq.cnpq.br;*
*isabelle.oswald@inra.fr*

Received: 2 November 2017 / Accepted: 11 December 2017
© 2018 Wageningen Academic Publishers

## Abstract

Mycotoxins are the most common contaminants of food and feed worldwide and are considered an important risk factor for human and animal health. Oxidative stress occurs in cells when the concentration of reactive oxygen species exceeds the cell's antioxidant capacity. Oxidative stress causes DNA damage, enhances lipid peroxidation, protein damage and cell death. This review addresses the toxicity of the major mycotoxins, especially aflatoxin $B_1$, deoxynivalenol, nivalenol, T-2 toxin, fumonisin $B_1$, ochratoxin, patulin and zearalenone, in relation to oxidative stress. It summarises the data associated with oxidative stress as a plausible mechanism for mycotoxin-induced toxicity. Given the contamination caused by mycotoxins worldwide, the protective effects of a variety of natural compounds due to their antioxidant capacities have been evaluated. We review data on the ability of vitamins, flavonoids, crocin, curcumin, green tea, lycopene, phytic acid, L-carnitine, melatonin, minerals and mixtures of anti-oxidants to mitigate the toxic effect of mycotoxins associated with oxidative stress.

**Keywords:** antioxidants, mycotoxins, oxidative stress.

# 1. Introduction

Mycotoxins are secondary fungal metabolites often found as contaminants in agricultural commodities all over the world and pose a risk for human and animal health (Bennett and Klich, 2003; Wu *et al.*, 2014a). More than 400 different mycotoxins have been isolated and chemically characterised. Those of major medical and agricultural concern are aflatoxins, fumonisins, ochratoxins, trichothecenes, zearalenone (ZEA) and patulin (PAT) (Wu *et al.*, 2014a).

The molecular mechanisms behind the toxic effects of the major mycotoxins are established and oxidative stress and the generation of free radicals have been shown to be implicated in mycotoxin toxicity (Adhikari *et al.*, 2017; Wang *et al.*, 2016). Indeed, the imbalance between free radicals and the antioxidant defence systems can cause chemical damage to DNA, proteins and lipids, as observed upon exposure to mycotoxins (Assi, 2017).

As human and animal exposure to mycotoxins is unavoidable, effective ways to mitigate their harmful impacts are required. Several studies have demonstrated the beneficial effects of antioxidant substances in the prevention and treatment of various diseases (Li *et al.*, 2015). In this context, the use of natural antioxidants has been shown to mitigate and/or prevent the toxic effects of mycotoxins (Sorrenti *et al.*, 2013).

The aims of this review are first to describe the cellular mechanisms involved in the physiological control and imbalance of free radical generation; second to summarise the toxic effects of the major mycotoxins associated with oxidative stress; and third, to present the main natural antioxidants used to mitigate the toxic effects of these mycotoxins.

# 2. Oxidative stress: physiological control and damage caused by overproduction of free radicals

One consequence of aerobic conditions is activation of oxidative mechanisms and the subsequent generation of reactive oxygen species (ROS) (Droge, 2002). Cells have developed primary and secondary enzymatic systems to avoid ROS-induced damage (Valko *et al.*, 2007). Superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) are characterised as primary antioxidant enzymes that trigger the breakdown of free radicals or combine toxic compounds with glutathione (GSH). The mechanisms of action of these enzymes are diverse. SOD breaks down the superoxide anion radical into $H_2O$ and $O_2$, whereas CAT catalyses the decomposition of hydrogen peroxide ($H_2O_2$) into water and oxygen. GPx reduces hydrogen peroxide to water and GR can regenerate GSH (Droge, 2002). By contrast, glutathione S-transferase (GST), a secondary detoxification enzyme, acts by binding ROS to GSH (Hayes and Strange, 1995) or by detoxifying lipid peroxides (Pickett and Lu, 1989).

Other mechanisms, including cysteine and GSH, are also involved in physiological control of ROS generation (Droge, 2002). GSH interacts with multiple antioxidant enzymes, modulating the action of GR, GPx and GST (a decrease in GSH content reduces enzymatic activity).

A control mechanism also exists for the expression of enzymes with antioxidant activity. Control is regulated by antioxidant response elements (AREs) that are activated by nuclear factor erythroid 2-related factor 2 (Nrf2) (Jin *et al.*, 2014). Nrf2-ARE is considered to be an important signalling pathway associated with antioxidant activity. Cells subjected to oxidative stress induce Nrf2 translocation to the nucleus, thereby activating genes encoding antioxidant enzymes and detoxifying enzymes of phase II (e.g. SOD) through ARE binding. Interestingly, although high antioxidant induction is associated with Nrf2 when this pathway is activated by ROS, the response is limited because ROS also activates a cell death-signalling pathway (Jin *et al.*, 2014; Valko *et al.*, 2007).

Organelles such as peroxisomes and mitochondria provide membrane-limited compartments specialised in redox activities. Consumption of oxygen leads to the production of $H_2O_2$, which oxidises some molecules. Furthermore, these organelles contribute to metabolic functions as they contain CAT, an enzyme that decomposes the $H_2O_2$ and prevents intracellular accumulation of this compound (Valko *et al.*, 2007). Cellular respiration in mitochondria creates one of the main superoxide production sites. During this process, ATP is produced by the electron transport chain and during energy transduction, free radical superoxide is formed, which has been associated with the cell pathophysiology in several diseases (Droge, 2002; Valko *et al.*, 2007).

ISSN 1875-0710 print, ISSN 1875-0796 online, DOI 10.3920/WMJ2017.2267

## Generation of oxidative stress, free radicals, and damage to DNA, proteins and lipids

Cells in homeostasis may produce free radicals as a result of physiological reactions (cellular respiration, for example). A variety of exogenous factors can promote oxidative stress and overproduction of free radicals (Young and Woodside, 2001). Oxidative stress occurs in cells when the production of ROS, such as such as hydroxyl radical (HO), perhydroxyl radical ($HOO^-$), superoxide anion ($O_2^-$) and reactive nitrogen species (RNS) including nitric oxide (NO), exceeds the antioxidant capacity of a cell (Valko *et al.*, 2007). Changes in intracellular antioxidant systems or in the production of free radicals can result in oxidative stress (Halliwell and Whiteman, 2004). Increased ROS production alters and/or activates several intracellular mechanisms that promote oxidative damage to DNA, proteins and membrane lipids. Lipid peroxidation may also lead to cell death. The mechanisms involved in the induction of cell apoptosis caused by the generation of ROS include activation of p53, mitogen-activated protein kinases (MAPKs), caspases and changes in the Bcl-2/Bax expression (Farley *et al.*, 2006).

Cells in homeostasis are maintained in a redox state through the association of the iron and copper redox couple. However, in a situation of oxidative stress, when superoxide is overproduced, the 'free iron' ($Fe^{2+}$) is released into the cytoplasm. This release considerably increases the oxidative stress, and leads to the generation of other reactive radicals through the Fenton reaction. In this reaction, $Fe^{2+}$ and $H_2O_2$ generate one of the most harmful radicals, the reactive hydroxyl ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH^{+}OH^{-}$). Transition metal ions, mainly iron, have been implicated in the generation of highly reactive radicals leading to DNA and membrane damage. Cellular and organelle membranes are attractive targets for oxidation due to the polyunsaturated fatty acid residues of phospholipids (Birben *et al.*, 2012). Secondary ROS metabolites can be produced, including endoperoxides (cyclisation reaction) and malondialdehyde (MDA), the toxic final product of lipid peroxidation, which is potentially mutagenic (Birben *et al.*, 2012; Marnett, 1999).

The level of cytosolic calcium ($Ca^{2+}$) can be increased by ROS generation through an influx of extracellular $Ca^{2+}$ or mobilisation of intracellular $Ca^{2+}$ stores (Droge, 2002). This increase in the cytosolic $Ca^{2+}$ level contributes to the activation of protein kinase C alpha and to the transcriptional induction of the activator protein 1 (AP1), c-Fos and c-Jun (Maki *et al.*, 1992). MAPK signalling cascades are activated through a variety of membrane receptors (receptor tyrosine kinases, protein tyrosine kinases, receptors of cytokines and growth factors, and heterotrimeric G protein-coupled receptors) and are regulated by phosphorylation and dephosphorylation on serine and/or threonine residues (Droge, 2002). The association between oxidative stress and the generation of free radicals can activate the MAPK pathway, mainly c-Jun N-terminal kinase (JNK) and p38, resulting in cell apoptosis (Allen and Tressini, 2000).

Another important free reactive radical produced in biological systems is NO. NO is a normal cellular metabolite with different functions in cells including neurotransmission, maintaining vascular tone, defence, smooth muscle relaxation and immune regulation (Bergendi *et al.*, 1999). Like with ROS, a nitrosative stress occurs following overproduction of RNS and disruption of the antioxidant system (Ridnour *et al.*, 2004). The mechanisms of cell damage induced by nitrosative stress include changes in protein structure (through nitrosylation reactions) leading to inhibition of their function (Valko *et al.*, 2007) and cell apoptosis. The induction of apoptosis by NO is associated with a decrease in the concentration of cardiolipin, an important component of the inner mitochondrial membrane. This molecule contributes to the optimal function of enzymatic systems involved in mitochondrial energy metabolism. A decrease in the cardiolipin level results in disruption of the electron transport chain, changes in mitochondria permeability and the release of cytochrome C into the cytosol (Droge, 2002). Furthermore, free radicals, such as superoxide anion and NO, are produced by phagocytic cells during the respiratory burst occurring in the inflammatory process. Together, these radicals can react to produce the peroxynitrite anion ($ONOO^{-}$), a molecule that is a powerful oxidant and can cause DNA fragmentation and lipid oxidation.

The generation of free radicals can increase the expression of cyclooxygenase-2 (COX-2), and of arachidonic acid metabolism, promote the upregulation of proinflammatory cytokines, such as tumour necrosis factor (TNF), interleukin(IL)-1, IL-6 and IL-8, thereby inducing a chronic inflammatory response and the stimulation of more free radicals (Reuter *et al.*, 2010). Extensive data has shown that oxidative stress contributes to the inflammatory process, which, in turn, leads to overproduction of reactive radical species thereby promoting a harmful feedback process that increases cellular damage.

The primary function of the respiratory chain is to use the energy produced to transfer electrons into the mitochondrial intermembrane space. However, a small percentage of electrons escape from the mitochondrial space, producing superoxide (Birben *et al.*, 2012). In normal conditions, the production of superoxide is

limited by SOD, which transforms the anion into hydrogen peroxide (Droge, 2002). Under oxidative stress, overproduction of superoxide occurs via activation of nicotine adenine dinucleotide phosphate (NADPH) and depletion of SOD (Birben *et al.*, 2012). Under oxidative stress, some organelles including peroxisomes, mitochondria and endoplasmic reticulum (ER) are affected by the overproduction of free radicals, mainly associated with lipid peroxidation. The peroxisome damage leads to CAT depletion and intracellular accumulation of $H_2O_2$ (Valko *et al.*, 2007). The mitochondria are an important target for injury induced by oxidative stress caused via endogenous metabolic processes and/or exogenous oxidative influences (Guo *et al.*, 2013). The mitochondrial damage to DNA caused by oxidative stress can result in a decrease in proteins that are important for electron transport, leading to the generation of ROS and the dysregulation of organelles, which, in turn, activate cell apoptotic mechanisms (Van Houten *et al.*, 2006). Furthermore, radicals such as $NO^-$ and $ONOO^-$ are responsible for detrimental changes in the mitochondrial respiratory chain (Sas *et al.*, 2007). The structural changes in mitochondrial proteins result in altered function in which enzymatic systems of the electron-transport chain (nicotinamide adenine dinucleotide dehydrogenase, cytochrome-c-oxidase, and adenosine triphosphate synthase) are the main targets of the free radicals (Van Houten *et al.*, 2006).

ROS can also alter mitochondrial phospholipids resulting in lipid peroxidation, which, in turn, increases mitochondrial membrane permeability. The mitochondrial permeability transition pore (MPTP) can be induced by ROS generation due the oxidation of thiol groups on the adenine nucleotide translocator (part of the MPTP) (Valko *et al.*, 2007). $ONOO^-$ can also affect mitochondrial homeostasis and energy production by inactivating enzymatic systems and promoting the release of mitochondrial $Ca^{2+}$ (Douarre *et al.*, 2012). The intracellular elevation of the $Ca^{2+}$ level also changes mitochondrial membrane potential (MMP) and induces the production of superoxide radicals, resulting in a vicious cycle (Douarre *et al.*, 2012) The mitochondrial excess of $Ca^{2+}$ contributes with the formation of MPTP, to osmotic swelling and rupture of the outer mitochondrial membrane (Douarre *et al.*, 2012). These mitochondrial changes caused by oxidative stress can lead to cell apoptosis due to the release of cytochrome-c, changes in Bcl2/Bax expression (down-regulation of the Bcl2 protein and an increase in Bax expression), activation of MAPKs and casp3 (Anuradha *et al.*, 2001; Farley *et al.*, 2006).

The ER is an organelle that regulates protein synthesis, drug detoxification, carbohydrate metabolism, lipid biosynthesis and $Ca^{2+}$ homeostasis. Oxidative stress and ROS generation deregulate the ER functions and release $Ca^{2+}$ into the cytosol (Minasyan *et al.*, 2017). The ER and mitochondria interact physiologically and functionally at sites called mitochondrial associated membranes. The damage to ER caused by oxidative stress results in mitochondrial dysfunction and cell apoptosis (Kim *et al.*, 2008).

# 3. Response to mycotoxins and oxidative stress: interaction in *in vitro*, *in vivo* and *ex vivo* models

The mycotoxins aflatoxin $B_1$ ($AFB_1$), deoxynivalenol (DON), nivalenol (NIV), fumonisin $B_1$ ($FB_1$), ochratoxin A (OTA), PAT and ZEA are the main contaminants of food and feed worldwide and have been extensively studied due their toxic effects on the human and the animal health (Wu *et al.*, 2014a). Several aspects of the intracellular action of mycotoxins have been elucidated: the induction of oxidative stress and ROS generation have become one of the major triggers of their lesional mechanisms, as observed in *in vitro*, *in vivo* and *ex vivo* studies. The oxidative stress mechanisms associated with $AFB_1$, DON, NIV, T-2 toxin (T-2), $FB_1$, OTA, PAT and ZEA are summarised in Figure 1 and

## Aflatoxin $B_1$

Aflatoxins are fungal metabolites mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Saini and Kaur, 2012). More than 10 forms of aflatoxins are known, among which the main ones are $AFB_1$, and aflatoxins $B_2$

($AFB_2$), $G_1$ ($AFG_1$), $G_2$ ($AFG_2$), $M_1$ ($AFM_1$) and $M_2$ ($AFM_2$) (Kumar *et al.*, 2017). Aflatoxins are natural contaminants of cereals (maize, rice, oats, barley and sorghum), groundnuts, pistachio nuts, almonds, cottonseed and walnuts (Wu *et al.*, 2014a). Milk can be contaminated by $AFM_1$, which is a principal hydroxylated-$AFB_1$ metabolite biotransformed by hepatic cytochrome P450 in cows fed an $AFB_1$ contaminated diet (Bennet and Klick, 2003).

The toxicity of $AFB_1$ is mainly associated with the binding of bioactivated $AFB_1$-8,9-epoxide to cellular macromolecules, such as mitochondria, nuclear nucleic acids and nucleoproteins, with cytotoxic effects (Bennet and Klich, 2003).

Studies *in vitro* (Liu and Wang, 2016; Mary *et al.*, 2012; Wang *et al.*, 2017) and *in vivo* (Abdel-Wahhab and Aly, 2003; Shi *et al.*, 2015) demonstrated that oxidative stress plays a major role in the toxic effects of $AFB_1$. The main consequences of ROS generation induced by $AFB_1$ are damage to DNA (Wang *et al.*, 2017; Zhang *et al.*, 2015b)

and mitochondrial lesions (Liu and Wang, 2016) as summarised in Figure 1. $AFB_1$ uncouples mitochondrial oxidative phosphorylation, reduces MMP and induces mitochondrial permeability (Liu and Wang, 2016; Shi *et al.*, 2015). The mitochondrial alterations associated with oxidative stress activate cytochrome C, modulate Bcl2/ Bax gene expression and activate caspase 9 and caspase 3 (Liu and Wang, 2016; Mary *et al.*, 2017; Wang *et al.*, 2017) leading to cell apoptosis. Mary *et al.* (2017) also reported that hepatocytes treated with $AFB_1$ increase the expression

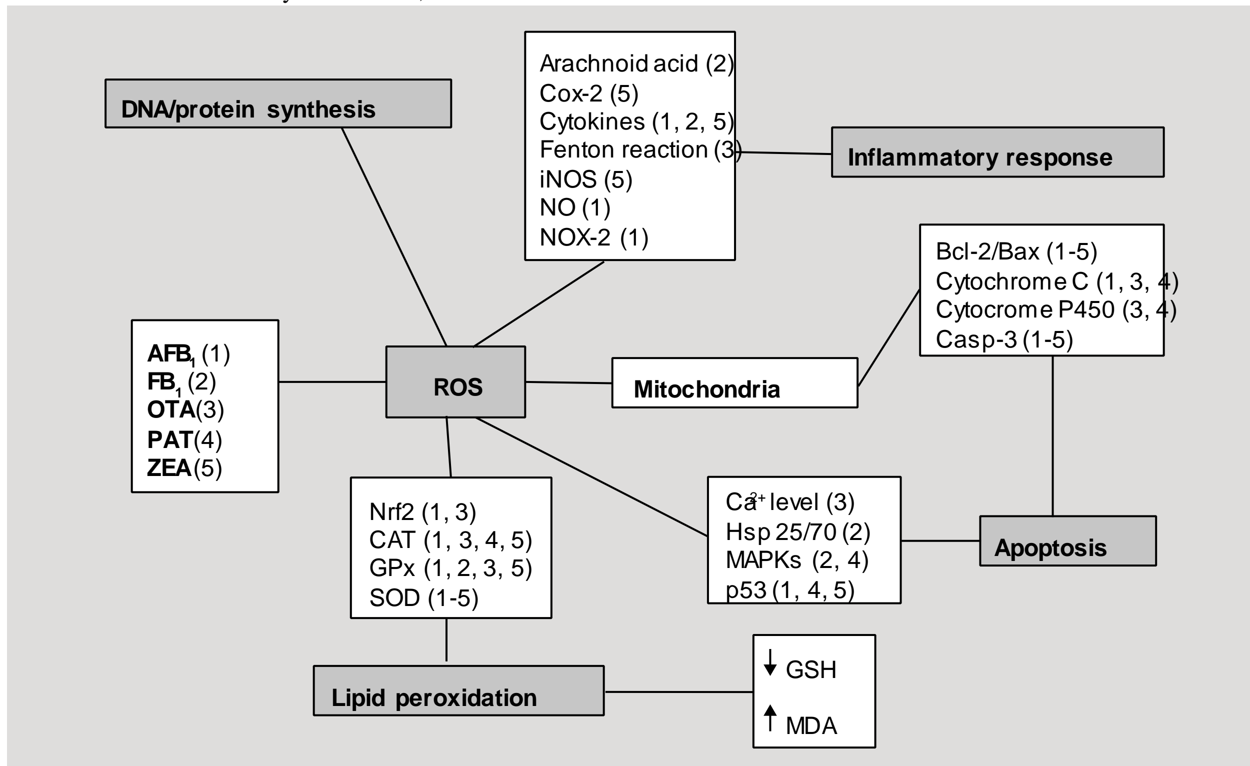



**Figure 1. Summary of the intracellular lesions associated with oxidative stress induced by the main mycotoxins that contaminate food and feed. $AFB_1$ = aflatoxin $B_1$; $FB_1$ = fumonisin $B_1$; OTA = ochratoxin A; PAT = patulin; ZEA = zearalenone. The numbers between brackets indicate the mycotoxins involved in each process.**

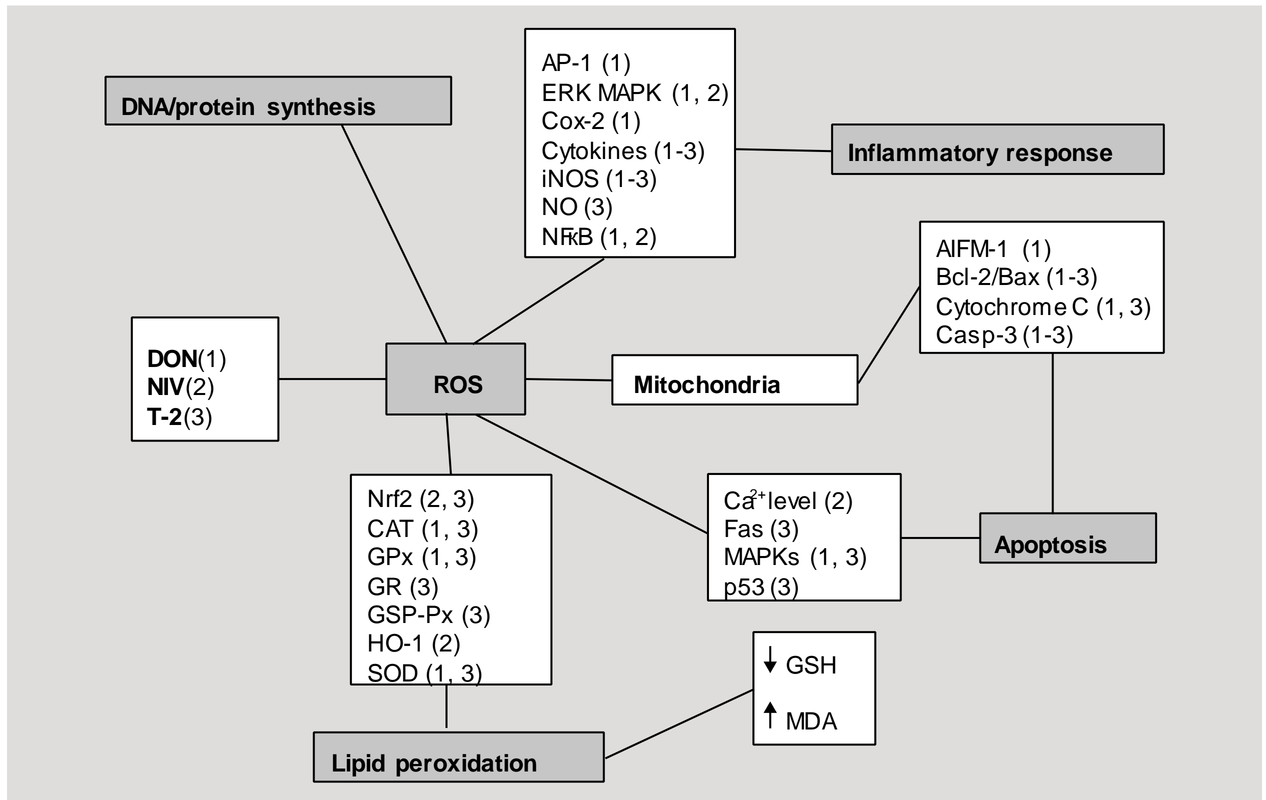



**Figure 2. Summary of the intracellular lesions associated with oxidative stress induced by trichothecenes that contaminate food and feed. DON = deoxynivalenol; NIV = nivalenol; T-2 = T-2-toxin. The numbers between brackets indicate the mycotoxins involved in each process.**

of the p53 gene, which was associated with an increase in cell apoptosis.

Recent studies have shown that $AFB_1$ causes changes in intracellular antioxidant mechanisms such as Nrf2, SOD, GPx and CAT expression (Liu and Wang *et al.*, 2016; Wang *et al.*, 2017), inhibiting antioxidant enzymes and causing an increase in lipid peroxidation (LPO) and a decrease in the level of GSH (Ma *et al.*, 2015; Maurya and Trigun, 2016). Moreover, ROS generation induced by $AFB_1$ modulates the inflammatory response through up-regulation of pro-inflammatory cytokines TNF-α, IL-1α, IL-1β and IL-6 and NO expression, by reducing anti-inflammatory cytokine IL-4 expression, inducing cytochrome P450 activity, increasing arachidonic acid metabolism, and activating the NADPH oxidase (NOX)2 dependent signalling pathway, thereby promoting the autophagy of pro-inflammatory macrophages M1 (An *et al.*, 2017; Ma *et al.*, 2015; Meissonier *et al.*, 2007)

## Deoxynivalenol

DON is a type B trichothecene predominantly produced by *Fusarium graminearum* and *Fusarium culmorum* (Bennet and Klich, 2003). Exposure to DON has been associated with alterations in the intestinal, immune, endocrine and nervous systems in several animal species and in humans (Maresca *et al.*, 2013; Payros *et al.*, 2016; Pestka, 2010a). At a molecular level, DON causes ribotoxic stress, inducing MAPK phosphorylation, promoting apoptosis, resulting in changes in the inflammatory response and decreasing the expression of cell adhesion proteins (Pierron *et al.*, 2016; Silva *et al.*, 2014).

Studies *in vitro* (Li *et al.*, 2014; Yang *et al.*, 2014; Zbynovska *et al.*, 2013) and *in vivo* (Borutova *et al.*, 2008; Osselaere *et al.*, 2013) established the toxic effects of DON associated with oxidative stress and ROS generation as observed in the Figure 2. DON alters the intracellular antioxidant defence system in target tissues such as liver, kidney, lymphoid organs, intestine and blood/serum as demonstrated by an increase in MDA concentration (Li *et al.*, 2014) and a decrease in GSH, SOD, CAT and GPx levels (Hou *et al.*, 2013; Strasser *et al.*, 2013; Zbynovska *et al.*, 2013).

The oxidative stress signalling pathway induced by DON has been suggested to be one of the mechanisms behind DNA fragmentation, cell death and apoptosis (Frankic *et al.*, 2008; Zhang *et al.*, 2009) as well as the

inhibition of protein synthesis and an increase in carbonyl content (Strasser *et al.*, 2013). Furthermore, alterations in the surface of lysosomal membranes lead to lysosomal fragility, a decrease in the MMP, an increase in membrane permeability and consequent deregulation of Bcl-2/Bax expression (leading to release of cytochrome C and activation of caspase 3, 8, 9 and apoptosis inducing factor mitochondrion associated 1) have been associated with ROS generation induced by DON (Kouadio *et al.*, 2005; Li *et al.*, 2014; Sun *et al.*, 2015). It has also been established that the ribotoxic stress induced by DON can stimulate apoptosis via activation of the p38 MAPK (Pestka *et al.*, 2008).

In addition, DON-induced oxidative stress can modulate the inflammatory response through up-regulation of pro-inflammatory cytokines including IL-1β, IL-2, IL6, IL-8, TNF-α, down-regulation of anti-inflammatory IL-4 and IL-10, selective activation of ERK MAPK, NFκB and AP-1, and increased and decreased expression of intracellular proteins involved in innate immunity, such as cyclooxygenase-2 (Cox-2) and inducible nitric oxide synthase (iNOS), respectively (Cano *et al.*, 2013; Graziani *et al.*, 2015; Pestka *et al.*, 2010b).

## Nivalenol

NIV is another type B trichothecene and is generally a biologically active metabolite of DON, present in agricultural commodities (Bennet and Klich, 2003). NIV is not as prevalent as DON, but NIV showed higher acute toxicity than DON (Alassane-Kpembi *et al.*, 2015; Cheat *et al.*, 2015). Studies *in vivo* (Cheat *et al.*, 2015), *in vitro* (Alassane-Kpembi *et al.*, 2015; Del Regno *et al.*, 2015; Marzocco *et al.*, 2009) and *ex vivo* (Alassane-Kpembi *et al.*, 2017; Cheat *et al.*, 2015) reported that NIV, such as DON, induce inhibition of protein, DNA and RNA synthesis, mitochondrial damage, cell apoptosis, decreases cellular viability and modulate inflammatory response mainly due to ROS generation associated with induction of oxidative stress as demonstrated in Figure 2, affecting the gastrointestinal tract and organs of the immune system.

The oxidative stress induced by NIV promotes ROS release via the NADPH oxidase signalling pathway, decreases the GSH level, alters $Ca^{2+}$ homeostasis and activates nuclear factor kappa beta (NF-κB) (Del Regno *et al.*, 2015). This ROS generation induces DNA and mitochondrial damage, activation of extracellular regulated kinase (ERK) MAPK, changes in Bcl-2 expression, up-regulation of Bax gene and activation of caspase 3, thereby promoting cell apoptosis (Marzocco *et al.*, 2009). The oxidative stress induced by NIV stimulates the antioxidant intracellular mechanisms of defence through an increase in heme oxygenase-1 (HO-1) and activation of Nrf2 (Del Regno *et al.*, 2015). In addition, the NIV-induced oxidative stress modulates the inflammatory response by activation of NF-κB, upregulation of pro-inflammatory cytokines such as IL-8, IL-1α, IL-1β, IL-17A, IL-22, interferon (IFN)-α and an increase in iNOS expression (Alassane-Kpembi *et al.*, 2017; Del Regno *et al.*, 2015; Marzocco *et al.*, 2009).

## T-2 toxin

T-2 is a type A trichothecene produced by several *Fusarium* species, mainly *Fusarium sporotrichiodes*, *Fusarium poae* and *Fusarium langsethiae*. Studies have demonstrated that T-2 affects the gastrointestinal tract, kidney, liver, heart, skin, the nervous, immunological, and reproductive systems, and embryogenic development in humans and animals (Agrawal *et al.*, 2012; Li *et al.*, 2011; Meissonnier *et al.*, 2008).

The main molecular target of trichothecenes is the ribosomal unit, affecting initiation of the polypeptide chain (Li *et al.*, 2011). Like other trichothecenes, T-2 binds and inactivates peptidyl transferase activity resulting in inhibition of protein synthesis and disruption of the mitochondrial morphology, ER and other membranes (Adhikari *et al.*, 2017). Studies *in vitro* (Chen *et al.*, 2008; Yang *et al.*, 2016; Zhang *et al.*, 2016) and *in vivo* (Chaudhari and Lakshmana, 2010) provided evidence that T-2-induced oxidative stress is associated with an increase in ROS generation and DNA, protein and lipid peroxidation leading to cell apoptosis.

The oxidative stress induced by T-2 promotes Fas upregulation, p53 activation, down-regulation of Bcl-2 and up-regulation of the pro-apoptotic factor Bax causing cytochrome C release, caspase 3 activation and cell apoptosis (Chen *et al.*, 2008; Zhang *et al.*, 2018) (Figure 2). ROS generation causes a decrease in Nrf2 expression, changes in the intracellular antioxidant enzymes GPx, GR, SOD and CAT, promoting a decrease in GSH level and an increase in MDA level (Wu *et al.*, 2014b; Yang *et al.*, 2016).

Another apoptosis signalling pathway linked to oxidative stress induced by T-2 is through the activation of JNK1, p38 MAPK, increase in heat shock protein (Hsp) 70 expression, increase in iNOS activity and NO release, causing mitochondrial damage and activation of caspase 3 (Chaudhari and Lakshmana, 2010; Li and Pestka, 2008). In addition, studies have shown that T-2 can modulate the inflammatory response by increasing the expression of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β and IL-11 (Agrawal *et al.*, 2012; Zhou *et al.*, 2014).

## Fumonisin $B_1$

Fumonisins are a group of mycotoxins mainly produced by *Fusarium verticillioides* and *Fusarium proliferatum* (Voss *et al.*, 2001). At least 15 related fumonisin compounds have been identified so far, but $FB_1$ is the most significant fumonisin due to its toxicity and widespread occurrence (Voss *et al.*, 2007).

At cellular level, $FB_1$ inhibits ceramide synthase, blocking the synthesis of sphingolipids, a class of membrane lipids that play an important role in cell signalling transduction pathways and cell growth, differentiation, and death (Grenier *et al.*, 2012; Voss *et al.*, 2007). Ceramide synthase inhibition leads to reduced levels of ceramide and intracellular accumulation of sphingolipids (So) and sphinganine (Sa). These free sphingoid bases are pro-apoptotic, cytotoxic growth inhibitors and are immunotoxic (Loiseau *et al.*, 2007; Voss *et al.*, 2001, 2007).

Studies *in vitro* (Domijan *et al.*, 2015; Mary *et al.*, 2012) and *in vivo* (Abbes *et al.*, 2016; Hassan *et al.*, 2015) revealed the potential of $FB_1$ to induce oxidative stress with consequent ROS generation, cytotoxic effects and apoptosis. The action of $FB_1$ on ROS generation has been considered a consequence rather than a mechanism of its toxicity (Galvano *et al.*, 2002; Wang *et al.*, 2016). However, some studies showed that $FB_1$ was able to increase the rate of oxidation, promote the production of free radicals and accelerate the chain reactions associated with lipid peroxidation in membranes (Hassan *et al.*, 2015; Stockmann-Juvala and Savolainen, 2008). These changes were demonstrated in different animal models by alterations in GPx and SOD expression, increase in MDA production and decrease in the GSH level (Abbes *et al.*, 2016; Domijan *et al.*, 2007; Poersch *et al.*, 2014).

The increase in ROS production induced by $FB_1$ has also been associated with inhibition of DNA synthesis and DNA fragmentation (Kouadio *et al.*, 2005; Wang *et al.*, 2016), inhibition of protein synthesis (Domijan *et al.*, 2007), mitochondrial injury with consequent deregulation of calcium homeostasis and caspase 3 activation, induction of cytochrome P450 activity with an increase in arachidonic acid metabolism and modulation of inflammatory response (Abbes *et al.*, 2016; Domijan and Abramov, 2011; Mary *et al.*, 2017). Some studies have demonstrated that perturbations of the cellular redox state due the $FB_1$ exposition can activate MAPKs and Hsp 25/70. Both signalling pathways can affect cell survival and are involved in the regulation of apoptosis (Lalles *et al.,* 2010; Rumora *et al.*, 2007) (Figure 1).

## Ochratoxin A

Ochratoxins are a group of mycotoxins produced by filamentous fungal species such as *Aspergillus* and *Penicillium* and occur in nature in three different isoforms: ochratoxin A, B and C. OTA is the most pathogenic to humans and animals, and is found in a wide range of foods and feed, including cereals, meat, dried fruits, nuts, coffee, wine and beer (Bennet and Klich, 2003; Limonciel and Jennings, 2014; Malir *et al.*, 2016).

Studies involving mammalian species *in vitro* (Bhat *et al.*, 2016; Gayathri *et al.*, 2015; Lautert *et al.*, 2014; Li *et al.*, 2015) and *in vivo* (Aydin *et al.*, 2003; Tanaka *et al.*, 2016) showed nephrotoxic, hepatotoxic, immunotoxic, enterotoxic, neurotoxic and teratogenic effects of OTA. The toxicity and carcinogenic mechanisms of OTA have been associated with induction of oxidative stress (Costa *et al.*, 2016), cell apoptosis (Ramyaa and Padma, 2013), cell autophagy/mitophagy (Gan *et al.*, 2017; Qian *et al.*, 2017) and protein synthesis inhibition (Mally and Dekant, 2009).

ROS generation has been reported to trigger OTA toxicity (Zhu *et al.*, 2017). Several oxidative stress mechanisms elicited by OTA have been proposed through *in vivo* (AbdelWahhab *et al.*, 2017; Gan *et al.*, 2017) and *in vitro* studies (Bhat *et al.*, 2016; Ramyaa *et al.*, 2014) (Figure 1). OTA can cause damage due to oxidative stress through the generation of hydroxyl radicals via the Fenton reaction, via flavoprotein NADPH-cytochrome P450 activation and inhibition of Nrf2 activation and gene transcription. In addition, OTA can decrease the expression of the intracellular antioxidant enzymes GPx, CAT, SOD and GR (Abdel-Wahhab *et al.*, 2017; Bhat *et al.*, 2016) as demonstrated by an increase in MDA levels.

ROS generation increased by OTA promotes the activation of the apoptosis signalling pathway through the mitochondrial lipid peroxidation, promoting loss of mitochondria membrane potential, increasing membrane permeability (Bhat *et al.*, 2016), activating JNK MAPKs (Zhu *et al.*, 2017) and affecting the ER calcium channels with consequent release of the calcium into cytosol (Sheu *et al.*, 2017). These lesional mechanisms promote changes in the Bcl-2 family, inducing the expression of Bax, facilitating the release of cytochrome C and the activation of caspase 3 in the cytosol.

## Patulin

PAT is a mycotoxin produced by several fungal species of the genera *Penicillium*, *Aspergillus*, *Paecilomyces* and *Byssochlamys* and is a common contaminant of apples and its products, rotten fruit, mouldy feed and stored cheese (Tannous *et al.*, in press).

The toxic effects of PAT have been described *in vitro* (Assunção *et al.*, 2016; Jayashree *et al.*, 2017; Zhang *et al.*, 2015a), *in vivo* (Boussabbeh *et al.*, 2016b; Lu *et al.*, 2017;) and *ex vivo* (Maidana *et al.*, 2016) mainly associated with ROS generation and activation of p53 protein and cleaved caspase 3 (Assunção *et al.*, 2016; Boussabbeh *et al.*, 2016b; Jayashee *et al.*, 2016; Jin *et al.*, 2016) (Figure 1).

PAT has a strong affinity for sulfhydryl groups (Tannous *et al.*, in press). Therefore, the rapid ROS generation observed in the PAT toxicity is likely due to its electrophilic attack of the intracellular antioxidant enzymes containing the sulfhydryl group, mainly GSH (Jin *et al.*, 2016). PAT decreases SOD and CAT activity, promoting an increase in MDA levels (Zhang *et al.*, 2015a).

ROS generation also leads to lipid peroxidation, modulation of p38 MAPK expression, injury of cellular membranes and consequent DNA damage (Jin *et al.*, 2016). The activation of p53 is initiated by ROS generation that results in an increase in ROS generation (feedback loop) due to the increase in p53-induced gene 3 (PIG 3) expression that induces the inhibition of anti-oxidant enzyme CAT (Jin *et al.*, 2016). In addition, p53 activation induces mitochondrial damage and caspase 3 activation leading to cell apoptosis (Boussabbeh *et al.*, 2016b). PAT also modulates other mechanisms associated with apoptosis regulation: it decreases Bcl-2 expression and increases Bax, cytochrome C and P450 expression (Boussabbeh *et al.*, 2016b; Jin *et al.*, 2016). Some studies have demonstrated that the generation of ROS causes mitochondrial damage and activates caspase 3 due to ER stress induced by PAT (Boussabbeh *et al.*, 2015, 2016a).

## Zearalenone

ZEA is a resorcylic acid lactone derived mycotoxin produced by *Fusarium* fungi and is a contaminant commonly found in unprocessed maize kernels. ZEA and its metabolites (α- and β-zearalenol) have structural analogy to oestrogens. The oestrogenic activity of ZEA and its derivative has been demonstrated both *in vivo* (Koraichi *et al.*, 2012) and *in vitro* (Frizzell *et al.*, 2011; Parveen *et al.*, 2009).

ZEA toxic effects can be induced by mechanisms that are not associated with its oestrogenic activity. ZEA affects the integrity of DNA and mitochondria, decreases cell proliferation and modulates the inflammatory response (Liu *et al.*, 2017; Marin *et al.*, 2015). These cytotoxic and genotoxic effects may be connected with oxidative stress generated by ZEA (Marin *et al.*, 2015). Some studies *in vivo* (Liu *et al.*, 2017; Marin *et al.*, 2015) and *in vitro* (Hassen *et al.*, 2007; Qin *et al.*, 2015) demonstrated the capacity of ZEA to induce ROS and lipid peroxidation, causing oxidative DNA and mitochondrial damage, apoptosis and modulation of pro- and anti-inflammatory cytokines as observed in Figure 1. The inhibition of protein and DNA synthesis caused by the oxidative stress was related to fragmentation of DNA, production of micronuclei and formation of DNA adduct (Abid-Essefi *et al.*, 2004). Furthermore, the decrease in cell proliferation could be the result of cell arrest in the G2/M phase induced by ZEA (Abid-Essefi *et al.*, 2003).

The generation of ROS by ZEA exposure led to an increase in iNOS and Cox-2 expression, and up-regulation of proinflammatory and down-regulation of anti-inflammatory cytokines (Marin *et al.*, 2015). Studies *in vivo* (Liu *et al.*, 2017; Marin *et al.*, 2015) and *in vitro* (Hassen *et al.*, 2007; Qin *et al.*, 2015) showed that ZEA also increases MDA levels due to the modulation of intracellular antioxidant mechanisms: decrease in GSH levels and SOD activity, increase in GPx and CAT activities. The latter enzymes are involved in intracellular antioxidant activity of the hydrogen peroxide conversion, consequently, the increase in GPx and CAT activities could be associated with an intracellular compensatory mechanism to scavenge ROS generation induced by ZEA (Marin *et al.*, 2015). Recent studies showed that ZEA-ROS generation increased the expression of p53, decreased MMP, promoting a decrease in anti-apoptotic Bcl-2 gene expression, leading to Bax expression and caspase 3 activation (Fan *et al.*, 2017). Therefore, the mitochondrial damage induced by the oxidative stress due to ZEA exposure can result in cell apoptosis.

## 4. Antioxidants and mycotoxins: does a protective effect exist?

Antioxidants are able to compete with other oxidisable substrates at relatively low concentrations, and thus to significantly delay or inhibit the oxidation of the substrates (Diplock *et al.*, 1998). The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, studies lemonstrated that the generation of oxidative stress f free radicals, mainly ROS and RNS, plays an ant role in the development of several diseases, including cancer (Reuter *et al.*, 2010; Zuo *et al.*, 2015). Similar protective action of antioxidants, mainly of

natural origin, has been observed against the toxic effects of several mycotoxins (Sorrenti *et al.*, 2013).

The protective properties of antioxidants are probably due to their ability to act as free radical scavengers, thereby protecting DNA, cell proteins and lipids from mycotoxininduced damage. Many natural substances have been used for their ability to modulate the oxidative stress caused by mycotoxins, including ascorbate (vitamin C), tocopherol (vitamin E), carotenoid (vitamin A) and the flavonoids (Diplock *et al.*, 1998; Sorrenti *et al.*, 2013; Strasser *et al.*, 2013). Several studies have also demonstrated the ability of crocin, curcumin, green tea, lycopene, phytic acid, L-carnitine, melatonin and minerals to modulate mycotoxin-induced oxidative stress (Meki *et al.*, 2004; Moosavi *et al.*, 2016; Salem *et al.*, 2016; Silva *et al.*, 2014; Verma and Mathuria, 2008; Zheng *et al.*, 2013).

## Vitamins

Vitamins, mainly vitamins A, C and E, and their precursors act as free radical scavengers. These vitamins reduce oxidative stress and mycotoxin-induced damage to the cells (Strasser *et al.*, 2013). The main effects of vitamins A, C and E on the cellular oxidative stress induced by mycotoxins observed in *in vitro* and *in vivo* studies are listed in Table 1.

**Table 1. Effects of antioxidant vitamins A, C and E in mycotoxin studies.**

| Mycotoxin[1] | Experimental model | Antioxidant effects[2] | Reference |
|---|---|---|---|
| Vitamin A | | | |
| $AFB_1$ | *in vitro*: human lymphocytes | increase GSH, GPx and SOD; decrease MDA | Alpsoy *et al.*, 2009 |
| | *in vitro*: microsomal enzymes | inhibit microsomal enzymes and reduce the bioactivation of $AFB_1$ | Wheeler *et al.*, 2006 |
| | *in vitro:* HepG2 cells | decrease the bioactivation of $AFB_1$; decrease apoptosis; inhibit p53 mutation | Reddy *et al.*, 2006 |
| | *in vivo*: mice | decrease the mitotic and meiotic clastogeny | Sinha and Dharmshila, 1994 |
| DON | *in vitro*: murine lymphoma cells | decrease the lipid (MDA) and protein peroxidation | Strasser *et al.*, 2013 |
| OTA | *in vivo:* rats | increase GSH and GPx; decrease apoptosis | Palabiyik *et al.*, 2013 |
| ZEA | *in vivo:* mice | decrease DNA adduct formation | Ghedira-Chekir *et al.*, 1998; Grosse *et al.*, 1997 |
| Vitamin C | | | |
| $AFB_1$ | *in vitro*: woodchuck hepatocytes | decrease DNA adduct formation | Yu *et al.*, 1994 |
| | *in vitro*: human lymphocytes | increase GSH, GPx and SOD; decrease MAD level | Alpsoy *et al.*, 2009 |
| | *in vitro*: microsomal enzymes | inhibit microsomal enzymes and reduce the bioactivation of $AFB_1$ | Wheeler *et al.*, 2006 |
| | *in vivo*: rats | increase $AFB_1$ metabolism to $AFM_1$ | Gradelet *et al.*, 1998 |
| | *in vivo*: guinea pig | decrease the GSH level; decrease the cytochrome P450 level | Netke *et al.*, 1997 |
| | *in vivo*: rabbits | decrease the number of abnormal and dead sperms | Salem *et al.*, 2001 |
| | *in vivo*: rohu (*Labeo rohita*) | increase serum lysozyme activity; enhance phagocytic ratio; immunostimulatory effect | Sahoo and Mukherjee, 2003 |
| DON | *in vitro*: rat erythrocytes | decrease the haemolytic effect | Rizzo *et al.*, 1992 |
| | *in vitro*: murine lymphoma cells | decrease the lipid (MDA) and protein peroxidation | Strasser *et al.*, 2013 |
| | *in vivo*: rats | increase the CAT, SOD and GST activities; increase GSH level; decrease MDA level | Atroshi *et al.*, 1995; Rizzo *et al.*, 1994 |
| OTA | *in vivo:* mice | decrease apoptosis | Atroshi *et al.*, 2000a |
| T-2 | *in vitro*: rat erythrocytes | decrease the haemolytic effect | Rizzo *et al.*, 1992 |
| | *in vivo*: rats | increase CAT, SOD, GST and GSH level | Atroshi *et al.*, 1995; Rizzo *et al.*, 1994 |
| ZEA | *in vivo:* piglets | increase T-AOC, SOD and GPx; decrease MDA level | Shi *et al.*, 2017 |

| | | | |
|---|---|---|---|
| | *in vivo:* mice | decrease DNA adduct formation | Ghedira-Chekir *et al.*, 1998; Grosse *et al.*, 1997 |
| Vitamin E | | | |
| AFB$_1$ | *in vitro*: HepG2 cells | decreased p53 mutation; decreased DNA adduct formation; decreased apoptosis | Reddy *et al.*, 2006; Abdel-Hamid and Firgany, 2015 |
| | *in vitro*: human lymphocytes | increased GST, GPx and SOD; decreased MDA level | Alpsoy *et al.*, 2009 |
| | *in vitro*: microsomal enzymes | inhibited of microsomal enzymes and reduce the bioactivation of AFB$_1$ | Wheeler *et al.*, 2006 |
| | *in vivo*: rats | increased the CAT, SOD and GST; decreased MDA level; decreased cytochrome P-450 activity | Cassandi *et al.*, 1993 |
| | *in vivo*: mice | increased 3β- and 17β-hydroxysteroid dehydrogenases activities and serum testosterone levels | Verma and Nair, 2002 |
| DON | *in vitro*: rat erythrocytes | decreased the haemolytic effect | Rizzo *et al.*, 1992 |
| | *in vitro*: murine lymphoma cells | decreased the lipid (MDA) and protein peroxidation | Strasser *et al.*, 2013 |
| | *in vivo*: piglets | decreased DNA damage | Frankic *et al.*, 2008 |
| | *in vivo*: rats | increased the CAT, SOD and GST and GSH level; decreased MDA level | Atroshi *et al.*, 1995; Rizzo *et al.*, 1994 |
| FB$_1$ | *in vivo*: rats | decrease DNA fragmentation; decreased the Ca$^{2+}$ nuclei; decreased the AST/ALT | Atroshi *et al.*, 1999 |
| OTA | *in vitro:* porcine fibroblasts | decreased DNA fragmentation | Fusi *et al.*, 2010 |
| | *in vitro:* HepG2 cells | decreased DNA fragmentation, Bax expression and casp-3 activation | Gayathri *et al.*, 2015 |
| | *in vivo*: rats | increase the protein level; decreased the AST, ALT, AP and γGT | Atroshi *et al.*, 2000b |
| | *in vivo:* mice | decreased apoptosis | Atroshi *et al.*, 2000a |

**Table 1. Continued.**

| Mycotoxin[1] | Experimental model | Antioxidant effects[2] | Reference |
|---|---|---|---|
| Vitamin E | | | |
| PAT | *in vitro*: HepG2 cells | decreased p53 activation; decreased DNA damage | Ayed-Boussema *et al.*, 2013 |
| T-2 | *in vitro*: chicken lymphocytes | increased lymphocyte proliferation | Jaradat *et al.*, 2006 |
| | *in vitro*: Vero cells | decreased Hsp 70 expression | El Golli *et al.*, 2006 |
| | *in vitro*: rat erythrocytes | decreased the haemolytic effect | Rizzo *et al.*, 1992 |
| | *in vivo*: chicken | decreased MDA level | Hoehler and Marquardt, 1996 |
| | *in vivo*: rats | increased the CAT, SOD and GST and GSH level; increase the protein level; decreased MDA level, AST, ALT, AP and γGT. | Atroshi *et al.*, 1995, 2000a.; Rizzo *et al.*, 1994 |
| ZEA | *in vivo*: mice | decreased DNA adduct formation | Ghedira-Chekir *et al.*, 1998; Grosse *et al.*, 1997 |

[1] AFB$_1$ = aflatoxin B$_1$; DON = deoxynivalenol; FB$_1$ = fumonisin B$_1$; NIV = nivalenol; OTA = ochratoxin A; PAT = patulin; T-2 = T-2 toxin; ZEA = zearalenone. [2] ALT = alanine transaminase; AP = alkaline phosphatase; AST = aspartate transaminase; CAT = catalase; GPx = glutathione peroxidase; GSH = glutathione; GST = glutathione S-transferase; γGT = gamma-glutamyl transpeptidase; MDA = malondialdehyde, SOD = superoxide dismutase; T-AOC = total antioxidative capacity.

Vitamin A has three active forms: retinol, retinal, and retinoic acid (retinoids), which are essential for physiological functions, including reproduction, vision, growth, and maintenance of epithelial tissues. The antioxidative effects of vitamin A have been associated with inhibiting cytochrome P450-mediated metabolism of toxic substances and preventing mutagenic epoxies from binding to DNA, thereby forming epoxides and competing with mutagenic epoxides in reaction with DNA (Diplock *et al.*, 1998). The toxic effects of $AFB_1$, DON, OTA and ZEA have been shown to be reduced in interaction with vitamin A in *in vitro* and *in vivo* models. The beneficial effects include increased levels of antioxidant enzymes (GSH, GPx), a decrease in mycotoxin bioactivation and in cell death (Table 1).

Vitamin C or ascorbic acid is a lactone synthesised in the liver of many species. It is a first-line antioxidant that has beneficial effects including protecting cell membranes, proteins and nucleic acids from oxidation. Its biological action and antioxidant characteristic are associated with its ability to donate electrons. At physiological levels, vitamin C is a powerful scavenger of oxygen-derived free radicals such as superoxide radical anion, $H_2O_2$, the hydroxyl radical, and singlet oxygen in plasma and tissues (Diplock *et al.*, 1998). In addition, ascorbic acid is an efficient scavenger of reactive nitrogen oxide species, thereby avoiding nitrosative stress and cell damage (Rock *et al.*, 1996). Vitamin C also interacts with GSH, reducing GSH production, which, in turn, reduces oxidative stress. The main effects of vitamin C on mycotoxin induced-toxicity are reducing lipid peroxidation and increasing levels of antioxidant enzymes. These and other effects have been described for $AFB_1$, DON, OTA, T-2 and ZEA (Table 1). Reduced adduct formation, decreased apoptosis and enhancement of phagocytosis have been reported for $AFB_1$ and ZEA (Ghedira-Chekir *et al.*, 1998; Sahoo and Mukherjee, 2003).

Vitamin E refers to a group of substances that includes tocols and tocotrienol derivatives. There are two forms of vitamin E, γ-tocopherol and α-tocopherol. α-tocopherol is the most biologically active form of vitamin E (Traber and Sies, 1996) and the major function is that of a peroxyl radical scavenger, interrupting the propagation of free radicals. In addition, vitamin E interacts with reactive nitrogen oxide species and singlet oxygen, thereby maintaining the integrity of polyunsaturated fatty acids in cell membranes (Rock *et al.*, 1996). Vitamin E has been shown to act favourably against seven mycotoxins ($AFB_1$, DON, $FB_1$, OTA, PAT, T-2 and ZEA) (Table 1). Its actions are similar to those of vitamins A and C, although decreased DNA fragmentation and damage of DON, $FB_1$ and OTA was also reported (Atroshi *et al.*, 1999; Frankic *et al.*, 2008; Gayathri *et al.*, 2015). In addition, reduced Hsp 70 expression and increased lymphocyte proliferation were described for T-2 (El Golli *et al.*, 2006; Jaradat *et al.*, 2006).

## Flavonoids

The flavonoids are the most common hydroxylated phenolic substances that are synthesised by plants. Sources of flavonoids are citrus fruits, berries, red wine and tea (Diplock *et al.*, 1998). The function of flavonoids is associated with its structure, which includes a number of structurally different subgroups, including flavonols (quercetin, kaempferol, myricetin), flavanols (catechin and epicatechin), isoflavones (genistein), flavones (apigenin, hesperetin), flavanones (naringenin, taxifolin) and/or anthocyanidins (cyanidin, malvidin) (Rice-Evans and Miller, 1996). The biological function of antioxidants is connected with their capacity to scavenger free radicals (peroxyl radical and hydroxyl radical), as well as chelating metals involved in the Fenton reaction (Rice-Evans and Miller, 1996).

The flavonol quercetin is one of the most effective polyphenolic substances linked to a reduction in the levels of ROS and reactive nitrogen species. In previous *in vivo* and *in vitro* studies, quercetin was shown to modulate the effects of oxidative stress caused by T-2, $AFB_1$ and OTA resulting in an increase in Nrf2 expression, SOD and GPx activity as well as total antioxidant status and GSH levels (Abdel-Wahhab *et al.*, 2017; Capcarova *et al.*, 2015; Choi *et al.*, 2010; Ramyaa and Padma, 2013; Ramyaa *et al.*, 2014). On the other hand, quercetin was associated with a decrease in ER oxidative stress, ROS generation, MAD level, P450 and NADPH activity, cytochrome C release, casp-3 activation cell apoptosis, Cox-2 and NO expression, TNF-α, IL-6 and IL-2 and a decrease in DNA damage (Abdel-Wahhab *et al.*, 2017; Ramyaa and Padma, 2013; Ramyaa *et al.*, 2014).

Recent studies demonstrated the antioxidant effects of other flavonoids on oxidative stress induced by mycotoxins. Proanthocyanidin increased Nrf2 expression, SOD, GPx, CAT activities, GSH level, and decreased MDA content, DNA damage and the expression of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and IFN-γ) in rats and mice subjected to $AFB_1$ (Long *et al.*, 2016c) and ZEA (Long *et al.*, 2016b) diets. Cyanidin decreased DNA damage, ROS production, lipid hydroperoxide and iNOS, and increased HO-1 activity and non-protein thiol groups in rats, in a pig kidney cell line (LLC-PK1), and in human fibroblasts exposed to OTA (Sorrenti *et al.*, 2012). Baicalein, wogonin and hesperidin increased cell viability and decreased genotoxicity and casp-3 activation in mice and neural crest cells

exposed to $AFB_1$ (Nones *et al.*, 2013; Ueng *et al.*, 2001). Zhong *et al.* (2017) reported that the apigenin re-established MMP, increased Bcl-2 expression, decreased Bax, p53 activation and the cytochrome C release in human embryonic kidney cells 293 (HEK 293 cells) treated with PAT. With the addition of silymarin to their diet, mice subjected to $FB_1$ contaminated feed showed decreased TNF-α expression and casp-8 activation (Sozmen *et al.*, 2014).

## Crocin, curcumin, green tea, lycopene and phytic acid

Crocin is a major bioactive compound and is mainly found in *Gardenia jasminoides* and saffron. Water and ethanol extracts of crocin displayed antioxidant activity against $O_2^-$ and HO radicals (Xiao *et al.*, 2017). Curcumin is a hydrophobic polyphenol derived from turmeric, a compound extracted from the root of *Curcuma longa* L. rhizome. Curcumin has diverse biological functions and its structure, which is composed of methoxy groups and phenols, is associated with its properties (Zheng *et al.*, 2017). The antioxidant action of crocin and curcumin on the molecular effects of mycotoxins *in vitro* and *in vivo* are summarised in Table 2.

Green tea is derived from *Camellia sinensis* leaves and contains a wide range of bioactive compounds of which one third are composed of polyphenols of which the majority are flavonoids. Catechins (GTCs) are one of the main flavonoids in green tea. GTCs have antioxidant capacity to scavenge ROS such as $O_2^-$, $H_2O_2$ and HO radicals (Cooper *et al.,* 2005). Lycopene is the most abundant carotenoid (nonvitamin A) in orange fruits and vegetables, mainly tomatoes and derived products and is responsible for their bright red colour. Lycopene is a recognised antioxidant and has been considered the most efficient in scavenging single oxygen (Mordente *et al.*, 2011). Phytic acid (IP6) is a saturated cyclic acid commonly found in plant tissues and seeds; and its antioxidant effect is on ROS production mainly due its capacity to chelate iron, thereby inhibiting the Fenton reaction (Silva and Bracarense, 2016). The antioxidant effects of green tea, lycopene and IP6 on mycotoxin-induced oxidative stress *in vitro* and *in vivo* studies are listed in Table 3.

## L-carnitine

L-carnitine is an endogenous mitochondrial membrane compound that plays a prominent role in facilitating the transport of long-chain fatty acids into mitochondria and the oxidation pathway (Adeva-Andany *et al.*, 2017). L-carnitine decreases oxidative stress, increases endogenous antioxidant defence capacity, protects mitochondria against lipid oxidation, and decreases apoptosis through the inhibition of mitochondrial swelling and cytochrome C release (AdevaAndany *et al.*, 2017). L-carnitine has been shown to decrease ROS production, MDA level, casp-3 activation, DNA and protein damage, and to increase MMP and GSH levels in rats and quails subjected to $AFB_1$ or T-2 contaminated diets (Citil *et al.*, 2005; Moosavi *et al.*, 2016; Yatim and Sachan, 2001).

## Melatonin

Melatonin is a hormonal product of the pineal gland that controls reproductive functions, modulates immune system activity, limits tumorigenesis and effectively inhibits oxidative stress (Reiter, 1997). Antioxidant effects of melatonin reported in rats exposed to $AFB_1$ and OTA contaminated diets included an increase in the GHS level and in the activity of CAT, GPx, GSH, GST, GR and SOD (Abdel-Wahhab *et al.*, 2005; Meki *et al.*, 2004; Sutken *et al.*, 2007), a decrease in MDA and LPO content (Abdel-Wahhab *et al.*, 2005; Sutken *et al.*, 2007; Yenilmez *et al.*, 2010) and decreased expression of NO, Hsp 70 and casp-3 (Meki *et al.*, 2001, 2004).

**Table 2. Effects of antioxidant food compounds (crocin and curcumin) in mycotoxins studies.**

| Mycotoxin[1] | Experimental model | Antoxidant effects[2] | Reference |
|---|---|---|---|
| Crocin | | | |
| $AFB_1$ | *in vivo*: rats | increase GSH level, GPx and GST activities; decrease DNA damage | Lin and Wang, 1986; Wang *et al.*, 1991 |
| PAT | *in vivo*: mice | increase GSH level and Bcl-2 expression; re-establish the MMP; decrease p53 activation, Bax expression and cytochrome C release; decrease MDA content, Hsp 70 expression, casp-3 activation and CAT; decrease DNA damage | Boubassabbeh *et al.*, 2016a,b |
| ZEA | *in vitro*: HCT11 and HEK293 cells | decrease MDA content, ER stress and DNA damage | Ben Salem *et al.*, 2015b |
| | *in vivo*: mice | increase Bcl-2 expression; decrease Bax, Hsp 70 expression and p53 activation; decrease MDA content, CAT and SOD activities; decrease protein carbonyl generation | Ben Salem *et al.*, 2015a; Salem *et al.*, 2016 |
| Curcumin | | | |
| $AFB_1$ | *in vitro*: human primary hepatocytes | decrease DNA damage | Gross-Steinmeyer *et al.*, 2009 |
| | *in vivo*: broiler chickens | increase GSH level, CAT, GST and SOD activities; decrease cytochrome P450 expression; decrease IL-6 expression | Gowda *et al.*, 2009; Yarru *et al.*, 2009 |
| | *in vivo*: mice | increase ATPase and SDH; increase GSH level, CAT, GPx and SOD activities; decrease DNA and protein damage | Mathuria and Verma, 2007a,b; Verma and Mathuria, 2008, 2009; Verma *et al.*, 2008 |
| | *in vivo*: rats | increase GSH level, CAT, SOD, GPx and GST activities; increase Bcl-2 expression; decrease Bax expression; decrease casp-3 activation; decrease DNA and protein adduct formation | Abdel-Wahhab *et al.*, 2016; El-Bahr, 2015; Nayak and Sashidhar, 2010; Poapolathep *et al.*, 2015 |
| ZEA | *in vivo*: rats | decrease MDA content; decrease DNA fragmentation | Ismaiel *et al.*, 2015 |
| | *in vivo*: sow | increase GSH level, CAT and SOD activities | Qin *et al.*, 2015 |

[1] $AFB_1$ = aflatoxin $B_1$; PAT = patulin; ZEA = zearalenone.

[2] ATPase = adenosine triphosphatase; CAT = catalase; GPx = glutathione peroxidase; GSH = glutathione; GST = glutathione S-transferase; GST = glutathione S-transferase; MDA = malondialdehyde; MMP = mitochondrial membrane potential; SDH = succinate dehydrogenase; SOD = superoxide dismutase.

## Minerals

Several minerals are dietary constituents involved in the antioxidant defence system, acting directly as antioxidants or promoting detoxifying enzymes (Sorrenti *et al.*, 2013). Antioxidant enzymes, such as GPx and SOD require a dietary supply of selenium (Se), copper (Cu) and zinc (Zn) (Wang *et al.*, in press).Se is an essential micronutrient associated with Sedependent enzymes, including GPx, thioredoxin reductases, iodothyronine deiodinases, and selenophosphate synthetases (Wang *et al.*, 2016). Se has been shown to increase the antioxidant function of CAT, GSH, GPx and SOD, decrease MDA content, increase the level of GSH and to modulate DON-induced immunosuppression in piglet lymphocytes and broiler chickens exposed to DON (Placha *et al.*, 2009; Wang *et al.*, in press). Long *et al.* (2016a,b) observed that Se increased GPx and SOD activities and Bcl-2 expression, decreased MDA content, Bax and casp-3 expression in mice fed with ZEA.

Zn exerts its antioxidant activity either in an acute way or on a long-term basis. In the first form, zinc acts by stabilising protein sulfhydryl groups against oxidation and exchanging redox active metals (copper and iron) (Zheng *et al.*, 2013). In the second form, Zn induces the expression of metallothioneins, which act as electrophilic scavengers. Moreover, zinc is a co-factor of the SOD enzyme that catalyses superoxide anions into less toxic $O_2$ and $H_2O_2$ and modulates the activity of GPx and glutamylcysteine synthetase, through the activation of metal response transcription factor-1 (MTF-1) (Powell, 2000). Zheng *et al.* (2013) demonstrated the antioxidant effect of zinc as being an increase in SOD activity and a decrease in ROS generation and in DNA damage in HepG2 cells exposed to OTA.

**Table 3. Effects of antioxidant food compounds (green tea/catechin, lycopene and phytic acid) in mycotoxins studies.**

| Mycotoxin[1] | Experimental model | Antoxidant effects[2] | Reference |
|---|---|---|---|
| Green tea/catechin | | | |
| $AFB_1$ | *in vitro*: chicken hepatocytes | increase Bcl2-expression; increase SOD, CAT, GR activities; decrease MDA content; decrease Bax and NF-κB expression; decrease TNF-α, IL-1β and IL-6 expression | Oskoueian *et al.*, 2015 |
| | *in vitro*: HepG2 | decrease ROS production | Corcuera *et al.*, 2012 |
| | *in vivo*: piglets | decrease the cytochrome 450 content | Tulayakul *et al.*, 2007 |
| | *in vivo*: rats | decrease the cytochrome 450 content; decrease DNA damage; decrease cell proliferation (promotion phase cancer) | Ito and Ito, 2007; Qin *et al.*, 2000 |
| $FB_1$ | *in vivo*: rats | increase GSH level | Marnewick *et al.*, 2009 |
| OTA | *in vitro*: LLC-PK1 cells | increase cell viability; decrease ROS production; decrease DNA damage | Costa *et al.*, 2007 |
| PAT | *in vivo*: mice | increase GSH level; decrease MDA content; decrease protein carbonyl formation; decrease p53 and casp-3 activation; decrease DNA damage | Jayashree *et al.*, 2017; Song *et al.*, 2014 |
| Lycopene | | | |
| $AFB_1$ | *in vivo*: rats | decrease DNA adduct formation | Tang *et al.*, 2007 |
| OTA | *in vivo*: rats | increase GSH level and GPx activity; decrease MDA content; decrease apoptosis; decrease DNA damage | Aydin *et al.*, 2013; Palabyik *et al.*, 2013 |
| T-2 | *in vivo*: chicks | increase GSH level; decrease MDA content | Leal *et al.*, 1999 |
| ZEA | *in vivo*: mice | increase GSH level; increase CAT, GPx, GST, GR and SOD activities; increase IL-10 expression; decrease TNF-α, IL-1β, IL-2 and IL-6 expression | Boeira *et al.*, 2015 |
| Phytic acid | | | |
| $AFB_1$ | *in vivo*: rats | increase GSH level; increase CAT and SOD activities | Abu El-Saad and Mahmoud, 2009 |
| DON | *in vitro*: IPEC-1 cells | increase TEER | Pacheco *et al.*, 2012 |
| | *ex vivo*: piglet intestine | decrease Cox-2 and casp-3 expression | Silva *et al.*, 2014 |
| $FB_1$ | *ex vivo*: piglet intestine | decrease Cox-2 and casp-3 expression | Silva *et al.*, 2014 |

[1] $AFB_1$ = aflatoxin $B_1$; $FB_1$ = fumonisin $B_1$; OTA = ochratoxin A; PAT = patulin; T-2 = T-2 toxin; ZEA = zearalenone.

[2] CAT = catalase; GPx = glutathione peroxidase; GR = glutathione reductase; GSH = glutathione; GST = glutathione S-transferase; IL = interleukin; MDA = malondialdehyde; NF-κB = nuclear factor kappa beta; ROS = reactive oxygen species; SOD = superoxide dismutase; TEER = transepithelial electrical resistance; TNF-α = tumour necrosis factor alpha

## Mixtures

Several studies have demonstrated that mixtures of natural substances reduce the oxidative stress lesions caused by mycotoxins. The combination of L-carnitine, vitamin E, selenium, melatonin, coenzyme Q10 and tamoxifen (Abidin *et al.*, 2013; Atroshi *et al.*, 2000; Sutken *et al.*, 2007; Yenilmez *et al.*, 2010) increased protective effects on DNA, proteins and lipids against OTA-induced toxicity compared to the individual effects of the compounds.

Moreover, the combination of coenzyme Q10, L-carnitine, alpha-tocopherol and selenium, garlic and curcumin (ElBarbary, 2016), black tea and curcumin (Alm-Eldeen *et al.*, 2015) displayed potent antioxidant effects against the toxic effects of $AFB_1$. In T-2-induced oxidative stress experiments, the increase in the level of GSH and the decrease in DNA damage were more apparent in mixtures of coenzyme Q10, L-carnitine, alpha-tocopherol and selenium (Atroshi *et al.*, 1999) and tamoxifen, vitamin E, and Se (Atroshi *et al.*, 1997, 2000).

## 5. Conclusions

Oxidative stress, ROS and RNS generation induced by mycotoxins have been associated with their cytotoxic effects on DNA, protein synthesis and mitochondria. These effects have been confirmed in different assays on cell membranes, proteins or nucleic acids, but the mechanisms involved in the activation of the signalling pathways that results in cell death or increased permeability for the different mycotoxins remain uncertain. Which factors are involved in activation? Dose, duration of exposure, and animal species are some of the aspects that need to be investigated in addition to the molecular characteristics of mycotoxins. In addition, most available data were acquired in *in vitro* studies or mice/rat models. New data from other animal models, especially those of economic interest are still lacking.

Several antioxidants have demonstrated their beneficial effects in mitigating and/or preventing the toxic effects of mycotoxins in *in vitro*, *in vivo* and *ex vivo* experimental models, but again the mechanisms and pathways involved in these effects are still not fully understood, pointing to a wide range of research opportunities. Although numerous studies have demonstrated the protective and preventive effect of antioxidants on mycotoxins -induced oxidative stress, the choice of the most appropriate nutritional methods requires knowledge of the type of antioxidants in the diet, their bioavailability and food sources, and the exact intake required to achieve these protective effects.

# References


Abbes, S., Ben Salah-Abbes, J., Jebali, R., Younes, R.B. and Queslati, R., 2016. Interaction of aflatoxin $B_1$ and fumonisin $B_1$ in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. Journal of Immunotoxicology 13: 46-54.

Abdel-Hamid, A.A.M. and Firgany, A.E.-D.L., 2015. Vitamin E supplementation ameliorates aflatoxin B1-induced nephrotoxicity in rats. Acta Histochemica 117: 767-779.

Abdel-Wahhab, M.A., Abdel-Galil, M.M. and El-Lithey, M., 2005. Melatonin counteracts oxidative stress in rats fed an ochratoxin A contaminated diet. Journal of Pineal Research 38: 130-135.

Abdel-Wahhab, M.A., Aljawish, A., El-Nekkety, A.A., Abdel-Aziem, S.H. and Hassan, N.S., 2017. Chitosan nanoparticles plus quercetin suppress the oxidative stress, modulate DNA fragmentation and gene expression in the kidney of rat fed ochratoxin A-contaminated diet. Food and Chemical Toxicology 99: 209-221.

Abdel-Wahhab, M.A., Salman, A.S., Ibrahim, M.I., El-Kady, A.A., Abdel-Aziem, S.H., Hassan, N.S. and Waly, A.I., 2016. Curcumin nanoparticles loaded hydrogels protects against aflatoxin $B_1$-induced genotoxicity in rat liver. Food and Chemical Toxicology 94: 159-171.

Abid-Essefi, S., Baudrimont, I., Hassen, W., Ouanes, Z., Mobio, T.A., Anane, R., Creppy, E.E. and Bacha, H., 2003. DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and Caco-2 cells: prevention by Vitamin E. Toxicology 192: 237-248.

Abid-Essefi, S., Ouanes, Z., Hassen, W., Baudrimont, I., Creppy, E. and Bacha, H., 2004. Cytotoxicity inhibition of DNA and protein syntheses and oxidative damage in cultured cells exposed to zearalenone. Toxicology *in vitro* 18: 467-474.

Abidin, Z., Khan, M.Z., Khatoon, A., Saleemi, M.K., Khan, A. and Javed, I., 2013. Ameliorative effects of L-carnitine and vitamin E (α-tocopherol) on haematological and serum biochemical parameters in White Leghorn cockerels given ochratoxin A contaminated feed. British Poultry Science 54: 471-477.

Abu El-Saad, A.S. and Mahmoud, H.M., 2007. Phytic acid exposure alters aflatoxinB1-induced reproductive and oxidative toxicity in albino rats (*Rattus norvegicus*). Evidence-Based Complementary and Alternative Medicine. 6: 332-341.

Adeva-Andany, M.M., Calvo-Castro, I., Fernandez-Fernandez, C., Donapetry-Garcia, C. and Pedre-Pineiro, A.M., 2017. Significance of L-carnitine for human health. IUBMB Life 69: 578-594.

Adhikari, M., Negi, B., Kaushik, N., Adhikari, A., Al-Khedhairy, A.A., Kaushik, N.K. and Choi, E.H., 2017. T-2 mycotoxin: toxicological effects and decontamination strategies. Oncotarget 8: 33933-33952.

Agrawal, M., Yadav, P., Lomash, V., Bhaskar, A.S. and Lakshmana Rao, P.V., 2012. T-2 toxin induced skin inflammation and cutaneous injury in mice. Toxicology 302: 255-265.

Alassane-Kpembi, I., Puel, O. and Oswald, I.P., 2015. Toxicological interactions between the mycotoxins deoxynivalenol, nivalenol and their acetyl derivatives in intestinal epithelial cells. Archives of Toxicology 89: 1337-1346.

Alassane-Kpembi, I., Puel, O., Pinton, P., Cossalter, A.-M., Chou, T.C. and Oswald, I.P., 2017. Co-exposure to low doses of the food contaminants deoxynivalenol and nivalenol has a synergistic inflammatory effect on intestinal explants. Archives of Toxicology 91: 2677-2687.

Allen, R.G. and Tressini, M., 2000. Oxidative stress and gene regulation. Free Radical Biology and Medicine 28: 463-499.

Alm-Eldeen, A.A., Mona, M.H., Shati, A.A. and El-Mekkawy, H.I., 2015. Synergistic effect of black tea and curcumin in improving the hepatotoxicity induced by aflatoxin $B_1$ in rats. Toxicology and Industrial Health 31: 1269-1280.

Alpsoy, L., Yildrim, A. and Agar, G., 2009. The antioxidant effects of vitamin A, C, and E on aflatoxin $B_1$-induced oxidative stress in human lymphocytes. Toxicology and Industrial Health 25: 121-127.

An, Y., Shi, X., Tang, X., Wan, Y., Shen, F., Zhang, Q., Wang, C., Jiang, M., Liu, M. and Yu, L., 2017. Aflatoxin $B_1$ induces reactive oxygen species-mediated autophagy and extracellular trap formation in macrophages. Frontiers in Cellular and Infection Microbiology 7: 53.

Anuradha, C.D., Kanno, S. and Hirano, S., 2001. Oxidative damage to mitochondria is a preliminary step to caspase-3 activation in fluoride-induced apoptosis in HL-60 cells. Free Radical Biology and Medicine 31: 367-373.

Assi, M., 2017. The differential role of reactive oxygen species in early and late stages of cancer. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 313: R646-R653.

Assunção, R., Alivto, P., Kleiveland, C.R. and Lea, T.E., 2016. Characterization of *in vitro* effects of patulin on intestinal epithelial and immune cells. Toxicology Letters 27: 250-251.

Atroshi, F., Biese, I., Saloniemi, H., Ali-Vehmas, T., Saari, S., Rizzo, A. and Veijalainen, P., 2000a. Significance of apoptosis and its relationship to antioxidants after ochratoxin A administration in mice. Journal of Pharmacy and Pharmaceutical Sciences 3: 281-291.

Atroshi, F., Rizzo, A., Biese, I., Lindberg, L.A. and Saloniemi, H., 1995. Effects of feeding T-2 toxin and deoxynivalenol on DNA and GSH contents of brain and spleen of rats supplemented with vitamin E and C and selenium combination. Journal of Animal Physiology and Animal Nutrition 74: 157-164.

Atroshi, F., Rizzo, A., Biese, I., Veijalainen, P., Antila, E. and Westermarck, T., 1997. T-2 toxin-induced DNA damage in mouse livers: the effect of pretreatment with coenzyme Q10 and alphatocopherol. Molecular Aspects of Medicine 18: S255-S258.

Atroshi, F., Rizzo, A., Biese, I., Veijalainen, P., Saloniemi, H., Sankari, S. and Andersson, K., 1999. Fumonisin $B_1$-induced DNA damage in rat liver and spleen: effects of pretreatment with coenzyme Q10, L-carnitine, alpha-tocopherol and selenium. Pharmacology Research 40: 459-467.

Atroshi, F., Rizzo, A., Sankari, S., Biese, I., Westermarck, T. and Veijalainen, P., 2000. Liver enzyme activities of rats exposed to ochratoxin A and T-2 toxin with antioxidants. Bulletin of Environmental Contamination and Toxicology 64: 586-592.

Aydin, G., Ozçelik, N., Ciçek, E. and Soyöz, M., 2003. Histopathologic changes in liver and renal tissues induced by ochratoxin A and melatonin in rats. Human and Experimental Toxicology 22: 383-391.

Aydin, S., Palabiyik, S.S., Erkekoglu, P., Sahin, G., Başaran, N. and Giray, B.K., 2013. The carotenoid lycopene protects rats against DNA damage induced by Ochratoxin A. Toxicon. 73: 96-103.

Ayed-Boussema, I., Abassi, H., Bouaziz, C., Hlima, W.B., Ayed, Y. and Bacha, H., 2013. Antioxidative and antigenotoxic effect of vitamin E against patulin cytotoxicity and genotoxicity in HepG2 cells. Environmental Toxicology 28: 299-306.

Ben Salem, I., Boussabbeh, M., Helali, S., Abid-Essefi, S. and Bacha, H., 2015a. Protective effect of crocin against zearalenone-induced oxidative stress in liver and kidney of Balb/c mice. Environmental Science and Pollution Research International 22: 19069-19076.

Ben Salem, I., Prola, A., Boussabbeh, M., Guilbert, A., Bacha, H., Abid-Essefi, S. and Lemaire, C., 2015b. Crocin and quercetin protect HCT116 and HEK293 cells from zearalenone-induced apoptosis by reducing endoplasmic reticulum stress. Cell Stress Chaperones 20: 927-938.

Bennett, J.W. and Klich, M., 2003. Mycotoxins. Clinical Microbiology Reviews 16: 497-516.

Bergendi, L., Benes, L., Durackova, Z. and Ferencik, M., 1999. Chemistry, physiology and pathology of free radicals. Life Science 65: 1865-1874.

Bhat, P.V., Pandareesh, M.D., Khanum, F. and Tamatam, A., 2016. Cytotoxic effects of ochratoxin A in neuro-2a cells: role of oxidative stress evidenced by N-acetylcysteine. Frontiers in Microbiology 7: 1142.

Birben, E., Sahiner, U.M., Sackese, C., Erzurum, S. and Kalayci, O., 2012. Oxidative stress and antioxidant defense. World Allergy Organisation Journal 5: 9-19.

Boeira, S.P., Funck, V.R., Borges Filho, C., Del'Fabbro, L., de Gomes, M.G., Donato, F., Royes, L.F., Oliveira, M.S., Jesse, C.R. and Furian, A.F., 2015. Lycopene protects against acute zearalenone-induced oxidative, endocrine, inflammatory and reproductive damages in male mice. Chemico-Biological Interactions. 230: 50-57.

Borutova, R., Faix, S., Placha, I., Gresakova, L., Cobanova, K. and Leng, L., 2008. Effects of deoxynivalenol and zearalenone on oxidative stress and blood phagocytic activity in broilers. Archives of Animal Nutrition 62: 303-312.

Boussabbeh, M., Ben Salem, I., Belquesmi, F., Bacha, H. and AbidEssefi, S., 2016a. Tissue oxidative stress induced by patulin and protective effect of crocin. Neurotoxicolgy 53: 343-349.

Boussabbeh, M., Ben Salem, I., Belquesmi, F., Neffati, F., Najjar, M.F., Abi-Essefi, S. and Bacha, H., 2016b. Crocin protects the liver and kidney from patulin-induced apoptosis *in vivo*. Environmental Science and Pollution Research International 23: 9799-9808.

Boussabbeh, M., Ben Salem, I., Neffati, F., Najjar, M., Bacha, M. and Abid-Essefi, S., 2015. Crocin prevents patulin-induced acute toxicity in cardiac tissues via the regulation of oxidative damage and apoptosis. Journal of Biochemical and Molecular Toxicology 29: 479-488.

Cano, P., Seeboth, J., Meurens, F., Cognie, J., Abrami, R., Oswald, I.P. and Guzylack-Piriou, L., 2013. Deoxynivalenol as a new factor in the persistence of intestinal inflammatory diseases: an emerging hypothesis through possible modulation of Th17-mediated response. PLoS ONE 8: e53647.

Capcarova, M., Petruska, P., Zbynovska, K., Kolesarova, A. and Sirotkin, A.V., 2015. Changes in antioxidant status of porcine ovarian granulosa cells after quercetin and T-2 toxin treatment. Journal of Environmental Science and Health, Part B 50: 201-206.

Cassand, P., Decoudu, S., Levegue, F., Daubeze, M. and Narbonne, J.F., 1993. Effect of vitamin E dietary intake on *in vitro* activation of aflatoxin $B_1$. Mutation Research. 319: 309-316.

Chaudhari, M., Lakshmana, R.P.V., 2010. Brain oxidative stress after dermal and subcutaneous exposure of T-2 toxin in mice. Food and Chemical Toxicology 48: 3436-3442.

Cheat, S., Gerez, J.R., Cognie, J., Alassane-Kpembi, I., Bracarense, A.P.F.L., Raymond-Letron, I., Oswald, I.P. and Kolf-Clauw, M., 2015. Nivalenol has a greater impact than deoxynivalenol on pig jejunum mucosa *in vitro* on explants and *in vivo* on intestinal loops. Toxins 7: 1945-1961.

Chen, J.H., Cao, J.L., Chu, Y.L., Wang, Z.L., Yang, Z.T. and Wang, H.-L., 2008. T-2 toxin-induced apoptosis involved Fas, p53, Bcl-xL, Bcl2, Bax and caspase-3 signaling pathways in human chondrocytes. Journal of Zhejiang University Science B9: 455-463.

Choi, K.C., Chung, W.T., Kwon, J.K., Yu, J.Y., Jang, Y.S., Park, S.M., Lee, S.Y. and Lee, J.C., 2010. Inhibitory effects of quercetin on aflatoxin $B_1$-induced hepatic damage in mice. Food and Chemical Toxicology 48: 2747-2753.

Citil, M., Gunes, V., Atakisi, O., Ozcan, A., Tuzcu, M. and Dogan, A., 2005. Protective effect of L-carnitine against oxidative damage caused by experimental chronic aflatoxicosis in quail (Coturnix coturnix). Acta Veterinaria Hungarica 53: 319-324.

Cooper, R., Morre, D.J. and Morre, D.M., 2005. Medicinal benefits of green tea: Part I. Review of non-cancer health benefits. Journal of Alternative and Complementary Medicine 11: 521-528.

Corcuera, L.A., Amezqueta, S., Arbillaga, L., Vettorazzi, A., Tourino, S., Torres, J.L. and Lopez de Cerain, A., 2012. A polyphenol-enriched cocoa extract reduces free radicals produced by mycotoxins. Food and Chemical Toxicology 50: 989-995.

Costa, J.G., Saraiva, N., Guerreiro, P.S., Louro, H., Silva, M.J., Miranda, J.P., Castro, M., Batinic-Haberle, I., Fernandes, A.S. and Oliveira, N.G., 2016. Ochratoxin A-induced cytotoxicity, genotoxicity and reactive oxygen species in kidney cells: an integrative approach of complementary endpoints. Food and Chemical Toxicology 87: 65-76.

Costa, S., Utan, A., Cervellati, R., Speroni, E. and Guerra, M.C., 2007. Catechins: natural free-radical scavengers against ochratoxin A-induced cell damage in a pig kidney cell line (LLC-PK1). Food and Chemical Toxicology 45: 1910-1917.

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. and Milzani, A., 2006. Biomarkers of oxidative damage in human disease. Clinical Chemistry 52: 601-623.

Del Regno, M., Adesso, S., Popolo, A., Quaroni, A., Autore, G., Severino, L. and Marzocco, S., 2015. Nivalenol induces oxidatve stress and increases deoxynivalenol pro-oxidant effect in intestinal epithelial cells. Toxicology and Applied Pharmacology 285: 118-127.

Diplock, A.T., Charleux, J.L., Grozier-Willi, G., Kok, F.J., Rice-Evans, C. and Roberfroid, M., 1998. Functional food science and defence against reactive oxidative species. British Journal of Nutrition 80, Suppl. 1: S77-S112.

Domijan, A.M. and Abramov, A.Y., 2011. Fumonisin $B_1$ inhibits mitochondrial respiration and deregulates calcium homeostasis – implication to mechanism of cell toxicity. International Journal of Biochemistry and Cell Biology 43: 897-904.

Domijan, A.M., Gajski, G., Jovanovic, I.N., Geric, M. and Garaj Vrhovac, V., 2015. *In vitro* genotoxicity of mycotoxin ochratoxin A and fumonisin $B_1$ could be prevented by sodium copper chlorophylin-implication to their genotoxic mechanism. Food Chemistry 170: 455-462.

Domijan, A.M., Peraica, M., Vrdoljak, A.L., Radić, B., Zlender, V. and Fuchs, R., 2007. The involvement of oxidative stress in ochratoxin A and fumonisin $B_1$ toxicity in rats. Molecular Nutrition and Food Research 51: 1147.

Douarre, C., Sourbier, C., Dalla Rosa, I., Brata Das, B., Redon, C.E., Zhnag, H., Neckers, L. and Pommier, Y., 2012. Mitochondrial topoisomerase I is critical for mitochondrial integrity and cellular energy metabolism. PLoS ONE 7: e41094.

Droge, W., 2002. Free radicals in the physiological control of cell function. Physiology Review 82: 47-95.

El Golli, E., Hassen, W., Bouslimi, A., Bouaziz, C., Ladjimi, M.M. and Bacha, H., 2006. Induction of Hsp 70 in Vero cells in response to mycotoxins cytoprotection by sub-lethal heat shock and by vitamin E. Toxicology Letters 166: 122-130.

El-Bahr, S.M., 2015. Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin $B_1$. Phytotherapy Research 29: 134-140.

El-Barbary, M.I., 2016. Detoxification and antioxidant effects of garlic and curcumin in *Oreochromis niloticus* injected with aflatoxin $B_1$ with reference to gene expression of glutathione peroxidase (GPx) by RT-PCR. Fish Physiology and Biochemistry 42: 617-629.

Fan, W., Shen, T., Ding, Q., Lv, Y., Li, L., Huang, K. and Yan, L., 2017. Zearalenone induces ROS-mediated mitochondrial damage in porcine IPEC-J2 cells. Journal of Biochemical and Molecular Toxicology 31: e21944.

Farley, N., Pedraza-Alva, G., Serrano-Gomez, D., Nagaleekar, V., Aronshtam, A., Krahl, T., Thornton, T. and Rincon, M., 2006. P38 mitogen-activated protein kinase mediates the fas-induced mitochondrial death pathway in $CD8^+$ T cells. Molecular and Cellular Biology 26: 2118-2129.

Frankic, T., Salobir, J. and Rezar, V., 2008. The effect of vitamin E supplementation on reduction of lymphocyte DNA damage induced by T-2 toxin and deoxynivalenol in weaned pigs. Animal Feed Science and Technology 141: 274-286.

Frizzell, C., Ndossi, D., Verhaegen, S., Dahl, E., Eriksen, G., Sorlie, M., Ropstad, E., Muller, M., Elliott, C.T. and Connolly, L., 2011. Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis. Toxicology Letters 206: 210-217.

Fusi, E., Rebucci, R., Pecorini, C., Campagnoli, A., Pinotti, L., Saccone, F., Cheli, F., Purup, S., Sejrsen, K. and Baldi, A., 2010. Alphatocopherol counteracts the cytotoxicity induced by ochratoxin a in primary porcine fibroblasts. Toxins 2: 1265-1278.

Galvano, F., Russo, A., Cardile, V., Galvano, G., Vanella, A. and Renis, M., 2002. DNA damage in human fibroblasts exposed to fumonisin $B_1$. Food and Chemical Toxicology 40: 25-31.

Gan, F., Hou, L., Zhou, Y., Liu, Y., Huang, D., Chen, X. and Huang, K., 2017. Effect of ochratoxin A on ER stress, MAPK signaling pathway and autophagy of kidney and spleen in pigs. Environmental Toxicology 32: 2277-2286.

Gayathri, L., Dhivya, R., Dhanasekaran, D., Periasamy, V.S., Alshatwi, A.A. and Akbarsha, M.A., 2015. Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: *in vitro* study in HepG2 cell. Food and Chemical Toxicology 83: 151-163.

Ghedira-Chekir, L., Maaroufi, K., Zakhama, A., Ellouz, F., Dhouib, S., Creppy, E.E. and Bacha, H., 1998. Induction of a SOS repair system in lysogenic bacteria by zearalenone and its prevention by vitamin E. Chemico-Biological Interactions 113: 15-25.

Gowda, N.K., Ledoux, D.R., Rottinghaus, G.E., Bermudez, A.J. and Chen, Y.C., 2009. Antioxidant efficacy of curcuminoids from turmeric (*Curcuma longa* L.) powder in broiler chickens fed diets containing aflatoxin $B_1$. British Journal of Nutrition 102: 1629-1634.

Gradelet, S., Le Bon, A.M., Bergès, R., Suschetet, M. and Astorg, P., 1998. Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B1 metabolism. Carcinogenesis. 19: 403-411.

Graziani, F., Pujol, A., Nicoletti, C., Pinton, P., Armand, L., Di Pasquale, E., Oswald, I.P., Perrier, J. and Maresca, M., 2015. The food-associated ribotoxin deoxynivalenol modulates the expression of the inducible NO synthase by the human intestinal epithelium. Toxicological Science 145: 372-382.

Grenier, B., Bracarense, A.P., Schwartz, H.E., Trumel, C., Cossalter, A.M., Schatzmayr, G., Kolf-Clauw, M., Moll, W.D. and Oswald, I.P., 2012. The low intestinal and hepatic toxicity of hydrolyzed fumonisin $B_1$ correlates with its inability to alter the metabolism of sphingolipids. Biochemical Pharmacology 83: 1465-1473.

Grosse, Y., Chekir-Ghedira, L., Huc, A., Obrecht-Pflumio, S., Dirheimer, G., Bacha, H. and 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: 225-229.

Gross-Steinmeyer, K., Stapleton, P.L., Tracy, J.H., Bammler, T.K., Strom, S.C., Buhler, D.R. and Eaton, D.L., 2009. Modulation of aflatoxin $B_1$-mediated genotoxicity in primary cultures of human hepatocytes by diindolylmethane, curcumin, and xanthohumols. Toxicological Science 112: 303-310.

Guo, C., Sun, L., Chen, X. and Zhang, D., 2013. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regeneration Research 8: 2003-2014.

Halliwell, B. and Whiteman, M., 2004. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? British Journal of Pharmacology 142: 231-255.

Hassan, A.M., Abdel-Aziem, S.H., El-Nekeety, A.A. and AbdelWahhab, M.A., 2015. *Panax ginseng* extract modulates oxidative stress, DNA fragmentation and up-regulate gene expression in rats subcronically treated with aflatoxin B and fumonisin B. Cytotechnology 67: 861-871.

Hassen, W., Ayed-Boussema, I., Oscoz, A.A., Lopez De Cerain, A. and Bacha, H., 2007. The role of oxidative stress in zearalenone-mediated toxicity in Hep G2 cells: oxidative DNA damage, gluthatione depletion and stress proteins induction. Toxicology 232: 294-302.

Hayes, J. and Strange, R., 1995. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. Free Radical Research 22: 193-207.

Hoehler, D. and Marquardt, R.R., 1996. Influence of vitamins E and C on the toxic effects of ochratoxin A and T-2 toxin in chicks. Poultry Science 75: 1508-1515.

Hou, Y.J., Zhao, Y.Y., Xiong, B., Cui, X.S., Kim, N.H., Xu, Y.X. and Sun, S.C., 2013. Mycotoxin-containing diet causes oxidative stress in the mouse. PLoS One 8: e60374.

Ismaiel, A.A.M., El-Denshary, E.S., El-Nekeety, A.A., Al-Yamani, A.F., Gad, S.A., Hassan, N.S. and Abdel-Wahhab, M.A., 2015. Ameliorative effects of curcumin nanoparticles on hepatotoxicity induced by zearalenone mycotoxin. Global Journal of Pharmacology 9: 234-245.

Jaradat, Z.W., Viia, B. and Marquardt, R.R., 2006. Adverse effects of T-2 toxin on chicken lymphocytes blastogenesis and its protection with vitamin E. Toxicology 225: 90-96.

Jayashree, G.V., Krupashree, K., Rachitha, P. and Khanum, F., 2017. Patulin induced oxidative stress mediated apoptotic damage in mice, and its modulation by green tea leaves. Journal of Clinical and Experimental Hepatology 7: 127-134.

Jin, H., Yin, S., Song, X., Zhang, E., Fan, L. and Hu, H., 2016. P53 activation contributes to patulin-induced nephrotoxicity via modulation of reactive oxygen species generation. Scientific Reports 6: 24455.

Jin, J., Xiong, T., Hou, X., Sun, X., Liao, J., Huang, Z., Huang, M. and Zhao, Z., 2014. Role of Nrf2 activation and NF-κB inhibition in valproic acid induced hepatotoxicity and in diammonium glycyrrhizinate induced protection in mice. Food and Chemical Toxicology 73: 95-104.

84


Kim, I., Xu, W. and Reed, J.C., 2008. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nature Reviews Drug Discovery 7: 1013-1030.

Koraichi, F., Videmann, B., Mazallon, M., Benahmed, M., Prouillac, C. and Lecoeur, S., 2012. Zearalenone exposure modulates the expression of ABC transporters and nuclear receptors in pregnant rats and fetal liver. Toxicology Letters 211: 246-256.

Kouadio, J.H., Mobio, T.A., Baudrimont, I., Moukaha, S., Dano S.D. and Creppy, E.E., 2005. Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin $B_1$ in human intestinal cell line Caco-2. Toxicology 213: 56-65.

Kumar, P., Mahato, D.K., Kamlel, M., Mohanta, T.K. and Kang, S.G., 2017. Aflatoxins: a global concern for food safety, human health and their management. Frontiers in Microbiology 7: 2170.

Lallès, J.P., Lessard, M., Oswald, I.P. and David, J.C., 2010. Consumption of fumonisin $B_1$ for 9 days induces stress proteins along the gastrointestinal tract of pigs. Toxicon 55: 244-249.

Lautert, C., Ferreiro, L., Wolkmer, P., Paim, F.C., Da Silva, C.B., Jaques, J.A., Lopes, S.T. and Santurio, J.M., 2014. Individual *in vitro* effects of ochratoxin A, deoxynivalenol and zearalenone on oxidative stress and acetylcholinesterase in lymphocytes of broiler chickens. Springerplus 3: 506.

Leal, M., Shimada, A., Ruiz, F. and Gonzalez de Mejia, E., 1999. Effect of lycopene on lipid peroxidation and glutathione-dependent enzymes induced by T-2 toxin *in vivo*. Toxicology Letters 209: 1-10.

Li, D., Ye, Y., Lin, S., Deng, L., Fan, X., Zhang, Y., Deng, X., Li, Y., Yan., H. and Ma, H.Y., 2014. Evaluation of deoxynivalenol-induced toxic effects on DF-1 cells *in vitro*: cell cycle arrest, oxidative stress, and apoptosis. Environmental Toxicology and Pharmacology 37: 141-149.

Li, M. and Pestka, J.J., 2008. Comparative induction of 28S ribosomal RNA cleavage by ricin and the trichothecenes deoxynivalenol and T-2 toxin in the macrophage. Toxicological Science 105: 67-78.

Li, X., Gao, J., Huang, K., Qi, X., Dai, Q., Mei, X. and Xu, W., 2015. Dynamic changes of global DNA methylation and hypermethylation of cell adhesion-related genes in rat kidneys in response to ochratoxin A. World Mycotoxin Journal 8: 465-476.

Li, Y., Wang, Z., Beier, R.C., De Shen, J., De Smet, D., De Saeger, S. and Zhang, S., 2011. T-2 toxin, a trichothecene mycotoxins: review of toxicity, metabolism, and analytical methods. Journal of Agricultural and Food Chemistry 59: 3441-3453.

Limonciel, A. and Jennings, P., 2014. A review of the evidence that ochratoxin A is an Nrf2 inhibitor: implications for nephrotoxicity and renal carcinogenicity. Toxins 6: 371-379.

Lin, J.K. and Wang, C.J., 1986. Protection of crocin dyes on the acute hepatic damage induced by aflatoxin $B_1$ and dimethylnitrosamine in rats. Carcinogenesis 7: 595-599.

Liu, M., Zhu, D., Guo, T., Zhang, Y., Shi, B., Shan, A. and Chen, Z., 2018. Toxicity of zearalenone on the intestines of pregnant sows and their offspring and alleviation with modified halloysite nanotubes. Journal of the Science and Food Agriculture 98: 698-706.

Liu, Y. and Wang, W.J., 2016. Aflatoxin $B_1$ impairs mitochondrial functions, activates ROS generation, induces apoptosis, and involves in Nrf2 signal pathway in primary broiler hepatocytes. Animal Science Journal 87: 1490-1500.

Loiseau, N., Debrauwer, L., Sambou, T., Bouhet, S., Miller, J.D., Martin, P., Viadère, J.L., Pinton, P., Puel, O., Pineau, T., Tulliez, J., Galtier, P. and Oswald, I.P., 2007. Fumonisin $B_1$ exposure and its selective effect on porcine jenunal segment: sphingolipids, glycolipids and transepithelial-passage disturbance. Biochemical Pharmacology 74: 144-152.

Long, M., Yang, S., Wang, Y., Li, P., Zhang, Y., Dong, S., Chen, X., Guo, J., He, J., Gao, Z. and Wang, J., 2016a. The protective effect of selenium on chronic zearalenone-induced reproductive system damage in male mice. Molecules 21: E1687.

Long, M., Yang, S., Zhang, Y., Li, P., Han, J., Dong, S., Chen, X. and He, J., 2016b. Proanthocyanidin protects against acute zearalenoneinduced testicular oxidative damage in male mice. Environmental Science and Pollution Research International 24: 938-946.

Long, M., Zhang, Y., Li, P., Yang, S.H., Zhang, W.K., Han, J.X., Wang, Y. and He, J.B., 2016c. Intervention of grape seed pro anthocyanidin extract on the subchronic immune injury in mice induced by aflatoxin $B_1$. International Journal of Molecular Science 17: 516.

Lu, X., Zhang, E., Yin, S., Fan, L. and Hu, H., 2017. Methylseleninic acid prevents patulin-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress and inactivation of p53 and MAPKs. Journal of Agricultural and Food Chemistry 65: 5299-5305.

Ma, Q., Li, Y., Fan, Y., Zhao, L., Wei, H., Ji, C. and Zhang, J., 2015. Molecular mechanisms of lipoic acid protection against aflatoxin B1-induced liver oxidative damage and inflammatory responses in broilers. Toxins 7: 5435-5447.

Maidana, L., Gerez, J.R., El Khoury, R., Pinho, F., Puel, O., Oswald, I.P. and Bracarense, A.P.F.R.L., 2016. Effects of patulin and ascladiol on porcine intestinal mucosa: an *ex vivo* approach. Food and Chemical Toxicology 98: 189-194.

Maki, A., Berezesky, I.K., Fargnoli, J., Holbrook, N.J. and Trump, B.F., 1992. Role of $Ca^{2+}$ in induction of c-*fos*, c-*jun*, and c-*myc* mRNA in rat PTE after oxidative stress. FASEB Journal 6: 919-924.

Malir, F., Ostry, V., Pfohl-Leszkowicz, A., Malir, J. and Toman, J., 2016. Ochratoxin A: 50 years of research. Toxins 8: 191.

Mally, A. and Dekant, W., 2009. Mycotoxins and the kidney: modes of action for renal tumor formation by ochratoxin A in rodents. Molecular Nutrition and Food Research 53: 467-478.

Maresca, M., 2013. From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins 5: 784-820.

Marin, D.E., Pistol, G.C., Neagoe, I.V., Calin, L. and Taranu, I., 2015. Effects of zearalenone on oxidative stress and inflammation in weanling piglets. Food and Chemical Toxicology 58: 408-415.

85

Marnett, L.J., 1999. Lipid peroxidation-DNA damage by malondialdehyde. Mutation Research – Fundamental and Molecular Mechanisms of Mutagenesis 424: 83-95.

Marnewick, J.L., Van der Westhuizen, F.H., Joubert, E., Swanevelder, S., Swart, P. and Gelderblom, W.C., 2009. Chemoprotective properties of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) herbal and green and black (*Camellia sinensis*) teas against cancer promotion induced by fumonisin $B_1$ in rat liver. Food and Chemical Toxicology. 47: 220-229.

Mary, V.S., Arias, S.L., Otaiza, S.N., Velez, P.A., Rubinstein, H.R. and Theumer, M.G., 2017. The aflatoxin $B_1$-fumonin $B_1$ toxicity in BRL-3A hepatocytes is associated to induction of cytochrome P450 activity and arachidonic acid metabolism. Environmental Toxicology 32: 1711-1724.

Mary, V.S., Theumer, M.G., Arias, S.L. and Rubinstein, H.R., 2012. Reactive oxygen species sources and biomolecular oxidative damage induced by aflatoxin $B_1$ and fumonisin $B_1$ in rat spleen mononuclear cells. Toxicology 302: 299-307.

Marzocco, S., Russo, R., Bianco, G., Autore, G. and Severino, L., 2009. Pro-apoptotic effects of nivalenol and deoxynivalenol trichothecenes in J774A.1 murine macrophages. Toxicology Letters 89: 21-26.

Mathuria, N. and Verma R.J., 2007a. Ameliorative effect of curcumin on aflatoxin-induced toxicity in DNA, RNA and protein in liver and kidney of mice. Acta Poloniae Phamaceutica 64: 497-502.

Mathuria, N. and Verma, R.J., 2007b. Curcumin ameliorates aflatoxininduced lipid peroxidation in liver, kidney and testis of mice-an *in vitro* study. Acta Poloniae Phamaceutica 64: 413-416.

Maurya, B.K. and Trigun, S.K., 2016. Fisetin modulates antioxidant enzymes and inflammatory factors to inhibit aflatoxin $B_1$ induced hepatocellular carcinoma in rats. Oxidative Medicine and Cellular Longevity 2016: 1972793.

Meissonnier, G.M., Laffitte, J., Loiseau, N., Benoit, E., Raymond, I., Pinton, P., Cossalter, A.M., Bertin, G., Oswald I.P. and Galtier P., 2007. Selective impairment of drug-metabolizing enzymes in pig liver during subchronic dietary exposure to aflatoxin $B_1$. Food and Chemical Toxicology 45: 2145-2154.

Meissonnier, G.M., Laffitte, J., Raymond, I., Benoit, E., Cossalter, A.M., Pinton, P., Bertin, G., Oswald, I.P. and Galtier, P., 2008. Subclinical doses of T-2 toxin impair acquired immune response and liver cytochrome P450 in pigs. Toxicology 247: 46-54.

Meki, A.R., Abdel-Ghaffar, S.K. and El-Gibaly, I., 2001. Aflatoxin $B_1$ induces apoptosis in rat liver: protective effect of melatonin. Neuroendocrinology Letters 22: 417-426.

Meki, A.R., Esmail Eel, D., Hussein, A.A. and Hassanein, H.M., 2004. Caspase-3 and heat shock protein-70 in rat liver treated with aflatoxin $B_1$: effect of melatonin. Toxicon 43: 93-100

Minasyan, L., Sreekumar, P.G., Hinton, D.R. and Kannan, R., 2017. Protective mechanisms of the mitochondrial-derived peptide humanin in oxidative and endoplasmic reticulum stress in RPE cells. Oxidative Medicine and Cellular Longevity 2017: 1675230.

Moosavi, M., Rezaei, M., Kalantari, H., Behfar, A. and Varnaseri, G., 2016. L-carnitine protects rat hepatocytes from oxidative stress induced by T-2 toxin. Drug and Chemical Toxicology 39: 445-450.

Mordente, A., Guantario, B., Meucci, E., Silvestrini, A., Lombardi, E., Martorana, G.E., Giardina, B. and Bohm, V., 2011. Lycopene and cardiovascular diseases: an update. Current Medicinal Chemistry 18: 1146-1163.

Nayak, S. and Sashidhar, R.B., 2010. Metabolic intervention of aflatoxin $B_1$ toxicity by curcumin. Journal of Ethnopharmacology 127: 641-644.

Netke, S. P., Roomi, M.W., Tsao, C. and Niedzwiecki, A., 1997. Ascorbic acid protects guinea pigs from acute aflatoxin toxicity. Toxicology and Applied Pharmacology. 143: 429-435.

Nones, J., Nones, J. and Trentin, A.G., 2013. Flavonoid hesperidin protects neural crest cells from death caused by aflatoxin $B_1$. Cell Biology International 37: 181-186.

Oskoueian, E., Abdullah, N., Zulkifli, I., Ebrahimi, M., Karimi, E., Goh, Y.M., Oskoueian, A. and Shakeri, M., 2015. Cytoprotective effect of palm kernel cake phenolics against aflatoxin $B_1$-induced cell damage and its underlying mechanism of action. BMC Complementary and Alternative Medicine 15: 392.

Osselaere, A., Santos, R., Hautekiet, V., Backer, P.D., Chiers, K., Ducatelle, R. and Croubels, S., 2013. Deoxynivalenol impairs hepatic and intestinal gene expression of selected oxidative stress, tight junction and inflammation proteins in broiler chickens, but addition of an adsorbing agent shifts the effects to the distal parts of the small intestine. PLoS ONE 8: e69014.

Pacheco, G.D., Silva, C.A., Pinton, P., Oswald, I.P. and Bracarense, A.P., 2012. Phytic acid protects porcine intestinal epithelial cells from deoxynivalenol (DON) cytotoxicity. Experimental Toxicology Pathology 64: 345-347.

Parveen, M., Zhu, Y. and Kiyama, R., 2009. Expression profiling of the genes responding to zearalenone and its analogues using estrogenresponsive genes. FEBS Letters 583: 2377-2384.

Payros, D., Alassane-Kpembi, I., Pierron, A., Loiseau, N., Pinton, P. and Oswald, I.P., 2016. Toxicology of deoxynivalenol and its acetylated and modified forms. Archives of Toxicology 90: 2931-2957.

Pestka, J.J., 2008. Mechanisms of deoxynivalenol-induced gene expression and apoptosis. Food Additives and Contaminants, Part A 25: 1128-1140.

Pestka, J.J., 2010a. Deoxynivalenol mechanism of action, human exposure, and toxicological relevance. Archives of Toxicology 84: 663-679.

Pestka, J.J., 2010b. Deoxynivalenol-induced proinflammatory gene expression: mechanisms and pathological squelae. Toxins 2: 1300-1317.

Pickett, C.B. and Lu, A.Y.H., 1989. Glutathione S-transferases: gene structure, regulation, and biological activity. Annual Review of Biochemistry 58: 743-764.

Pierron, A., Mimoun, S., Murate, L.S., Loiseau, N., Lippi, Y., Bracarense, A.P.F.L., Schatzmayr, G., He, J., Zhou, T., Moll, W.D. and Oswald, I.P., 2016. Microbial biotransformation of DON: molecular basis for reduced toxicity. Scientific Reports 6: 29105.

Placha, I., Borutova, R., Gresakova, L., Petrovic, V., Faix, S. and Leng, L., 2009. Effects of excessive selenium supplementation to diet contaminated with deoxynivalenol on blood phagocytic activity and antioxidative status of broilers. Journal of Animal Physiology and Animal Nutrition 93: 695-702.

Poapolathep, S., Imsilp, K., Machii, K., Kumagai, S. and Poapolathep, A., 2015. The effects of curcumin on aflatoxin $B_1$-induced toxicity in rats. Biocontrol Science 20: 171-177.

Poersch, A.B., Trombetta, F., Braga, A.C.M., Boeira, S.P., Oliveira, M.S., Dilkin, P., Mallmann, C.A., Fighera, M.R., Royes, L.F.F. and Oliveira, M.S., 2014. Involvement of oxidative stress in subacute toxicity induced by fumonisin $B_1$ in broiler chicks. Veterinary Microbiology 174: 180-185.

Powell, S.R., 2000. The antioxidant properties of zinc. Journal of Nutrition 130: 1447S-1454S.

Qian, G., Lu, D., Hu, J., Gan, F., Hou, L., Chen, X. and Huang, K., 2017. Ochratoxin A-induced autophagy *in vitro* and *in vivo* promotes porcine circovirus type 2 replication. Cell Death and Disease 8: e2909.

Qin, G., Ning, Y. and Lotlikar, P.D., 2000. Chemoprevention of aflatoxin $B_1$-initiated and carbon tetrachloride-promoted hepatocarcinogenesis in the rat by green tea. Nutrition and Cancer 38: 215-222.

Qin, X., Cao, M., Lai, F., Yang, F.G.W., Zhang, X., Cheng, S., Sun, X., Qin, G., Shen, W. and Li, L., 2015. Oxidative stress induced by zearalenone in porcine granulosa cells and its rescue by curcumin *in vitro*. PLoS ONE 10: e0127551.

Ramyaa, P. and Padma, W., 2013. Ochratoxin-induced toxicity, oxidative stress and apoptosis ameliorated by quercetin-modulation of Nrf2. Food and Chemical Toxicology 62: 205-216.

Ramyaa, P., Krishnaswamy, R. and Padma, W., 2014. Quercetin modulates OTA-induced oxidative stress and redox signaling in HepG2 cells-up regulation of Nrf2 expression and down regulation of NF-κB and Cox-2. Biochemica et Biophysica Acta 1840: 681-692.

Reddy, L., Odhav, B. and Bhoola, K., 2006. Aflatoxin $B_1$-induced toxicity in HepG2 cells inhibited by carotenoids: morphology, apoptosis and DNA damage. Biological Chemistry 387: 87-93.

Reiter, R.J., 1997. Aging and oxygen toxicity: relation to changes in melatonin. Age 20: 201-213.

Reuter, S., Gupta, S.C., Chaturvedi, M.M. and Aggarwal, B.B., 2010. Oxidative stress, inflammation, and cancer: how are they linked? Free Radical Biology and Medicine 49: 1603-1616.

Rice-Evans, C. and Miller, N.J., 1996. Antioxidant activities of flavonoids as bioactive components of food. Biochemical Society Transactions 24: 790-795.

Ridnour, L.A., Thomas, D.D., Mancardi, D., Espey, M.G., Miranda, K.M., Paolocci, N., Feelisch, M., Fukutu, J. and Wink, D.A., 2004. The chemistry of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. Putting perspective on stressful biological situations. Biological Chemistry 385: 1-10.

Rizzo, A.F., Atroshi, F., Hirvi, T. and Saloniemi, H., 1992. The hemolytic activity of deoxynivalenol and T-2 toxin. Natural Toxicology 1: 106-110.

Rizzo, A.F., Atroshmi, F., Hotupa, A., Sankar, S. and Elovaam, E., 1994. Protective effect of antioxidants against free radical-mediated lipid peroxidation induced by DON or T-2 toxin. Journal of Veterinary Medicine 41: 81-90.

Rock, C.L., Jacob, R.A. and Bowen, P., 1996. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. Journal of the American Dietetic Association 96: 693-702.

Rumora, L., Domijan, A.M., Grubisic, T.Z. and Peraica, M., 2007. Mycotoxin fumonisin $B_1$ alters cellular redox balance and signalling pathways in rat liver and kidney. Toxicology 242: 31-38.

Sahoo, P.K. and Mukherjee, S.C., 2003. Immunomodulation by dietary vitamin C in healthy and aflatoxin $B_1$-induced immunocompromised rohu (*Labeo rohita*). Comparative Immunology, Microbiology and Infectious Diseases 26: 65-76.

Saini, S.S. and Kaur, A., 2012. Aflatoxin B1: toxicity, characteristics and analysis: mini review. Global Advanced Research Journal of Chemistry and Material Science 1: 63-70.

Salem, I.B., Boussabbeh, M., Neffati, F., Najjar, M.F., Abid-Essefi, S. and Bacha, H., 2016. Zearalenone-induced changes in biochemical parameters, oxidative stress and apoptosis in cardiac tissue: protective role of crocin. Human and Experimental Toxicology 35: 623-634.

Salem, M.H., Kamel, K.I., Yusef, M.I., Hassa, G.A. and El-Nouty, F.D., 2001. Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin $B_1$. Toxicology 162: 209-218.

Sas, K., Robotka, H., Toldi, J. and Vecsei, L., 2007. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. Journal of Neurological Science 257: 221-239.

Sheu, M.L., Shen, C.C., Chen, Y.S. and Chiang, C.K., 2017. Ochratoxin A induces ER stress and apoptosis in mesangial cells via NADPH oxidase-derived reactive oxygen species-mediated calpain activaton pathway. Oncotarget 8: 19376-19388.

Shi, B., Su, Y., Chang, S., Sun, Y., Meng, X. and Shan, A., 2017. Vitamin C protects the piglet liver against zearalenone induced oxidative stress by modulating expression of nuclear receptors PXR and CAR and their target genes. Food and Function. 8: 3675-3687

Shi, D.Y., Liao, S.Q., Guo, S.N., Li, H., Yang, M.M. and Tang, Z.X., 2015. Protective effects of selenium on aflatoxin $B_1$-induced mitochondrial permeability transition, DNA damage, and histological alterations in duckling liver. Biological Trace Element Research 163: 162-168.

Silva, E.O. and Bracarense, A.P.F.R.L., 2016. Phytic acid: from antinutritional to multiple protection factor of organic systems. Journal of Food Science 81: R135-R1362.

Silva, E.O., Gerez, J.R., Drape, T.C. and Bracarense, A.F.R.L., 2014. Phytic acid decreases deoxynivalenol and fumonisin $B_1$-induced changes on swine jejunal explants. Toxicology Reports 1: 284-292.

Sinha, S.P. and Dharmshila, K., 1994. Vitamin A ameliorates the genotoxicity in mice of aflatoxin $B_1$-containing *Aspergillus flavus* infested food. Cytobios 79: 85-95.

Song, E., Xia, X., Su, C., Dong, W., Xian, Y., Wang, W. and Song, Y., 2014. Hepatotoxicity and genotoxicity of patulin in mice, and its modulation by green tea polyphenols administration. Food and Chemical Toxicology 71: 122-127.

Sorrenti, V., Di Giacomo, C., Acquaviva, R., Barbagallo, I., Bognanno, M. and Galvano, F., 2013. Toxicity of ochratoxin A and its modulation by antioxidants: a review. Toxins 5: 1742-1766.

Sorrenti, V., Di Giacomo, C., Acquaviva, R., Bognanno, M., Grilli, E., D' Orazio, N. and Galvano, F., 2012. Dimethylarginine dimethylaminohydrolase/nitric oxide synthase pathway in liver and kidney: protective effect of cyanidin 3-O-β-D-glucoside on ochratoxin-A toxicity. Toxins 4: 353-363.

Sozmen, M., Devrim, A.K., Tunca, R., Bayezit, M., Dag, S. and Essiz, D., 2014. Protective effects of silymarin on fumonisin $B_1$-induced hepatotoxicity in mice. Journal of Veterinary Science 15: 51-60.

Stockmann-Juvala, H. and Savolainen, K., 2008. A review of the toxic effects and mechanisms of action of fumonisin $B_1$. Human and Experimental Toxicology 27: 799-809.

Strasser, A., Carra, M., Ghareeb, K., Awad, W. and Bohm, J,. 2013. Protective effects of antioxidants on deoxynivalenol-induced damage in murine lymphoma cells. Mycotoxin Research 29: 203-208.

Sun, L.H., Lei, M.Y., Zhang, N.Y., Gao, X., Li, C., Krumm, C.S. and Qi, D.S., 2015. Individual and combined cytotoxic effects of aflatoxin $B_1$, zearalenone, deoxynivalenol and fumonisin $B_1$ on BRL 3A rat liver cells. Toxicon 95: 6-12.

Sutken, E., Aral, E., Ozdemir, F., Uslu, S., Alatas, O. and Colak, O., 2007. Protective role of melatonin and coenzyme Q10 in ochratoxin A toxicity in rat liver and kidney. International Journal of Toxicology 26: 81-87.

Tanaka, T., Hasegwa-Baba, Y., Watanabe, Y., Mizukami, S., Kangawa, Y., Yoshida, T. and Shibutani, M., 2016. Maternal exposure to ochratoxin A targets intermediate progenitor cells of hippocampal neurogenesis in rat offspring via cholinergic signal downregulation and oxidative stress responses. Reproductive Toxicology 65: 113-122.

Tang, L., Guan, H., Ding, X. and Wang, J.S., 2007. Modulation of aflatoxin toxicity and biomarkers by lycopene in F344 rats. Toxicology and Applied Pharmacology. 219: 10-17.

Tannous, J., Keller, N.P., Atoui, A., Elkhoury, A., Lteif, R., Oswald, I.P. and Puel, O., in press. Secondary metabolism in *Penicillium expansum*: emphasis on recent advances in patulin research.
Critical Reviews in Food Science and Nutrition. DOI: https://doi. org/10.1080/10408398.2017.

Traber, M.G. and Sies, H., 1996. Vitamin E in humans: demand and delivery. Annual Review of Nutrition 16: 321-347.

Tulayakul, P., Dong, K.S., Li, J.Y., Manabe, N. and Kumagai, S., 2007. The effect of feeding piglets with the diet containing green tea extracts or coumarin on *in vitro* metabolism of aflatoxin $B_1$ by their tissues. Toxicon 50: 339-548.

Ueng, Y.F., Shyu, C.C., Liu, T.Y., Oda, Y., Lin, Y.L., Liao, J.F. and Chen, C.F., 2001. Protective effects of baicalein and wogonin against benzo[a]pyrene- and aflatoxin $B_1$-induced genotoxicities. Biochemical Pharmacology 62: 1653-1660.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology 39: 44-84.

Van Houten, B., Woshner, V. and Santos, J.H., 2006. Role of mitochondrial DNA in toxic responses to oxidative stress. DNA Repair 5: 145-152.

Verma, R.J. and Nair, A., 2002. Effect of aflatoxins on testicular steroidogenesis and amelioration by vitamin E. Food and Chemical Toxicology. 40: 669-672.

Verma, R.J. and Mathuria, N., 2008. Curcumin ameliorates aflatoxininduced lipid-peroxidation in liver and kidney of mice. Acta Poloniae Phamaceutica 65: 195-202.

Verma, R.J. and Mathuria, N., 2009. Effect of curcumin on aflatoxininduced biochemical changes in testis of mice. Fertility and Sterility 91: 597-601.

Verma, R.J., Chakraborty, B.S., Patel, C. and Mathuria, N., 2008. Curcumin ameliorates aflatoxin-induced changes in SDH and ATPase activities in liver and kidney of mice. Acta Poloniae Pharmaceutica 65: 415-419.

Voss, K.A., Riley, R.T., Norred, W.P., Bacon, C.W., Filmore, I.M., Howard, P.C., Plattner, R.D., Collins, T.F.X. and Hansen, J.K., 2001. An overview of rodent toxicities: liver and kidney effects of fumonisins and *Fusarium moniliform*. Environmental Health Perspectives 109, Suppl. 2: 259-266.

Voss, K.A., Smith, G.W. and Hascheck, W.M., 2007. Fumonisins: toxicokinetics, mechanism of action and toxicity. Animal Feed Science and Technology 137: 299-325.

Wang, C.J., Shiow, S.J., Lin, J.K., 1991. Effects of crocetin on the hepatotoxicity and hepatic DNA binding of aflatoxin $B_1$ in rats. Carcinogenesis 12: 459-462.

Wang, W.J., Xu, Z.L., Yu, C. and Xu, X.H., 2017. Effects of aflatoxin $B_1$ on mitochondrial respiration, ROS generation and apoptosis in broiler cardiomyocytes. Animal Science Journal 2017: 1561-1567.

Wang, X., Wu, Q., Wan, D., Liu, Q., Chen, D., Liu, Z., MatinezLarranaga, M.R., Martinez, M.A., Anadon, A. and Yuan, Z., 2016. Fumonisins: oxidative stress-mediated toxicity and metabolism *in vivo* and *in vitro*. Archives of Toxicology 90: 81-101.

Wang, X., Zuo, Z., Deng, J., Zhang, Z., Chen, C., Fan, Y., Peng, G., Cao, S., Hu, Y., Yu, S., Chen, C. and Ren, Z., in press. Protective role of selenium in immune-relevant cytokine and immunoglobulin production by piglet splenic lymphocytes exposed to deoxynivalenol. Biological Trace Element Research. DOI: https://doi.org/10.1007/ s12011-017-1160-6.

Wheeler, J.L., Harrison, M.A. and Koehler, P.E., 2006. *In vitro* metabolism of aflatoxin $B_1$ with microsomal enzymes in the presence of selected nutrients. Journal of Food Science 52: 1432-1433.

Wu, F., Groopman, J.D. and Pestka, J.J., 2014a. Public health impacts of foodborne mycotoxins. Annual Review of Food Science and Technology 5: 351-372.

Wu, Q.H., Wang, X., Yang, W., Nüssler, A.K., Xiong, L.Y., Kuca, K., Dohnal, V., Zhang, X.J. and Yuan, Z.H., 2014b. Oxidative stress-mediated cytotoxicity and metabolism of T-2 toxin and deoxynivalenol in animals and humans: an update. Archives of Toxicology 88: 1309-1326.

Xiao, W., Li, S., Wang, S. and Ho, C.T., 2017. Chemistry and bioactivity of *Gardenia jasminoides*. Journal of Food and Drug Analysis 25: 43-61.

Yang, L., Yu, Z., Hou, J., Deng, Y., Zhou, Z., Zhao, Z. and Cui, J., 2016. Toxicity and oxidative stress induced by T-2 toxin and HT-2 toxin in broilers and broiler hepatocytes. Food and Chemical Toxicology 87: 128-137.

Yang, W., Yu, M., Fu, J., Bao, W., Wang, D., Hao, L., Yao, P., Nussler, A.K., Yan, H. and Liu, L., 2014. Deoxynivalenol induced oxidative stress and genotoxicity in human peripheral blood lymphocytes. Food and Chemical Toxicology 64: 383-396.

Yarru, L.P., Settivari, R.S., Gowda, N.K., Antoniou, E., Ledoux, D.R. and Rottinghaus, G.E., 2009. Effects of turmeric (*Curcuma longa*) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. Poultry Science 88: 2620-2627.

Yatim, A.M. and Sachan, D.S., 2001. Carnitine alters binding of aflatoxin to DNA and proteins in rat hepatocytes and cell-free systems. Journal of Nutrition 131: 1903-1908.

Yenilmez, A., Isikli, B., Aral, E., Degirmenci, I., Sutken, E. and Baycu, C., 2010. Antioxidant effects of melatonin and coenzyme Q10 on oxidative damage caused by single-dose ochratoxin A in rat kidney. Chinese Journal of Physiology 53: 310-317.

Young, I.S. and Woodside, J.V., 2001. Antioxidants in health and disease. Journal of Clinical Pathology 54: 176-186.

Yu, M.W., Zhang, Y.J., Blaner, W.S.and Santella, R.M., 1994. Influence of vitamins A, C, and E and beta-carotene on aflatoxin $B_1$ binding to DNA in woodchuck hepatocytes. Cancer 73: 596-604.

Zbynovska, K., Petruška, P. and Capcarova, M., 2013. Effect of deoxynivalenol on some haematological, biochemical and antioxidant parameters of porcine blood *in vitro*. Journal of Microbiology, Biotechnology and Food Sciences 2: 1611-1628.

Zhang, B., Peng, X., Li, G., Xu, Y., Xia, X. and Wang, Q., 2015a.
Oxidative stress is involved in patulin induced apoptosis in HEK 293 cells. Toxicon 94: 1-7.

Zhang, J., Zheng, N., Liu, J., Li, F.D., Li, S.L. and Wang, J.Q., 2015b. Aflatoxin $B_1$ and aflatoxin $M_1$ induced cytotoxicity and DNA damage in differentiated and undifferentiated Caco-2 cells. Food and Chemical Toxicology 83: 54-60.

Zhang, X., Jiang, L., Geng, C., Cao, J. and Zhong, L., 2009. The role of oxidative stress in deoxynivalenol-induced DNA damage in HepG2 cells. Toxicon 54: 513-518.

Zhang, Y., Han, J., Zhu, C.C., Tang, F., Cui, X.-S., Kim, N.-H. and Sun, S.-C., 2016. Exposure to HT-2 toxin causes oxidative stress induced apoptosis/autophagy in porcine oocytes. Scientific Reports 6: 33904.

Zhang, Y.F., Su, P.K., Wang, L.J., Zheng, H.Q., Bai, F.B., Li, P., Meng, X.P. and Yang, J.Y., 2018. T-2 toxin induces apoptosis via the baxdependent caspase-3 activation in mouse primary Leydig cells. Toxicology Mechanisms and Methods 28: 23-28.

Zheng, J., Zhang, Y., Xu, W., Luo, Y., Hao, J., Shen, X.L., Yang, X., Li, X. and Huang, K., 2013. Zinc protects HepG2 cells against the oxidative damage and DNA damage induced by ochratoxin A. Toxicology and Applied Pharmacology 268: 123-131.

Zheng, Q.T., Yang, Z.H., Yu, L.Y., Ren, Y.Y., Huang, Q.X., Liu, Q., Ma, X.Y., Chen, Z.K., Wang, Z.B. and Zheng, X., 2017. Synthesis and antioxidant activity of curcumin analogs. Journal of Asian Natural Products Research 19: 489-503.

Zhong, Y., Jin, C., Gan, J., Wang, X., Shi, Z., Xia, X. and Peng, X., 2017. Apigenin attenuates patulin-induced apoptosis in HEK293 cells by modulating ROS-mediated mitochondrial dysfunction and caspase signal pathway. Toxicon 137: 106-113.

Zhou, X., Wang, Z., Chen, J., Wang, W., Song, D., Li, S., Yang, H., Xue, S. and Chen, C., 2014. Increased levels of IL-6, IL-1beta, and TNF-alpha in Kashin-Beck disease and rats induced by T-2 toxin and selenium deficiency. Rheumatology International 34: 995-1004.

Zhu, L., Zhang, B., Dai, Y., Li, H. and Xu, W., 2017. A review: epigenetic mechanism in ochratoxin A toxicity studies. Toxins 9: 113.

Zuo, L., Zhou, T., Pannell, B.K., Ziegler, A.C. and Best, T.M., 2015. Biological and physiological role of reactive oxygen species – the good, the bad and the ugly. Acta Physiologica 214: 329-348.

# 3 OBJETIVOS

## 3.1 OBJETIVO GERAL

- Avaliar os efeitos tóxicos da micotoxinas fumonisina B1 (FB1) e desoxinivalenol (DON), e o efeito modulador do ácido fítico (IP6) sobre explantes jejunais de suínos.

## 3.2 OBJETIVOS ESPECÍFICOS

- Determinar os efeitos da FB1 e DON sobre a morfologia intestinal, apoptose, proliferação celular, expressão de E-caderina, ciclooxigenase-2 (cox-2) e estresse oxidativo.

- Caracterizar os efeitos do IP6 nos explantes jejunais expostos a FB1 e ao DON sob avaliação dos parâmetros morfológicos, proliferação celular, apoptose, expressão E-caderina, (cox-2) e estresse oxidativo.

- Avaliar os efeitos da FB1 e DON sobre a expressão de RNAm para as citocinas pró-inflamatórias (IL-1β, IL-6, IL-8, IFN-γ, TNF-α), anti-inflamatória (IL-10), e β-defensinas 1 (pBD-1) e 2 (pBD-2).

- Avaliar os efeitos do IP6 na resposta inflamatória por meio da avaliação da expressão de RNAm para as citocinas pró-inflamatórias (IL-1β, IL-6, IL-8, IL-10, IFN-γ, TNF-α) e anti-inflamatória (IL-10), e na expressão das β-defensinas 1 e 2 em explantes jejunais expostos a FB1 e DON.

## 4 ARTIGO 1

### PHYTIC ACID DECREASES THE OXIDATIVE STRESS AND THE INTESTINAL LESIONS INDUCED BY FUMONISIN B1 AND DEOXYNIVALENOL IN SWINE[2]

**Abstract**

The purpose of the present study was to investigate the effects of phytic acid (IP6) on morphological and immunohistochemical parameters, and on the oxidative stress response of intestinal explants of pigs exposed to fumonisin B1 (FB1) and deoxynivalenol (DON) alone and in combination. The jejunal explants were exposed to treatments: control, IP6 5mM, DON 10µM, FB1 70 µM, DON 10µM plus FB1 70 µM, DON 10µM + IP6 5mM, FB1 70 µM + IP6 5mM and DON 10µM plus FB1 70 µM + IP6 5 mM. Decrease of villi height and goblet cells density were more evident in DON and DON plus FB1 treatments. In addition, a significant increase in cell apoptosis and cell proliferation, and a decrease of E-cadherin expression were observed in the same groups. Mycotoxin exposure increased ciclooxigenase-2 (Cox-2) expression and decreased the cellular antioxidant capacity. Increase in lipid peroxidation was observed in DON and FB1 treated groups. IP6 showed beneficial effects such as reduction on intestinal morphological changes, cell apoptosis, cell proliferation and Cox-2 expression, and an increase in E-cadherin expression when compared with DON, FB1 alone or associated. IP6 inhibited the oxidative stress and increased the antioxidant capacity in the explants exposed to mycotoxins.

**Keywords:** Mycotoxins, IP6, reactive oxygen species, morphology, jejunum, pigs

---

[1]Manuscrito editado de acordo com as normas de publicação da Food and Chemical Toxicology (https://www.sciencedirect.com/journal/food-and-chemical-toxicology)

## Introduction

The mycotoxins fumonisin B1 (FB1) and deoxynivalenol (DON) are the most frequently contaminants in agricultural commodities worldwide, and represent a risk for human and animal health (Escrivá et al., 2015). The exposure to mycotoxins is inevitable, therefore, there is a requirement of effective approaches to mitigate or even eliminate their harmful impacts. In the last years, research involving nutraceutical substances and compounds is constantly increasing due to their excellent preventive and therapeutic effects (Adhikari et al., 2017).

FB1 and DON are mycotoxins mainly produced by *Fusarium* spp. that commonly contaminate maize, wheat, barley and oat (Alshannaq and Yu, 2017). The intracellular action of these mycotoxins has been elucidated, and the induction of oxidative stress and generation of radical oxygen species (ROS) play an important role in their toxic effects as observed *in vivo* (Osselaere et al., 2013; Abbes et al., 2016) and *in vitro* (Li et al., 2014; Domijan et al., 2015). Other toxic effects have been reported altering the intestinal morphology (expression of cell junction proteins, cell proliferation and apoptosis, production of mucin) and modulating the inflammatory response (deregulation of anti and pro-inflammatory cytokines and overexpression of ciclooxigenase-2 (cox-2) (Pinton et al., 2010; Bracarense et al., 2012; Basso et al., 2013; Wan et al., 2013; Silva et al., 2014). However, the association between intestinal lesions and oxidative stress induced by FB1 and DON has not been elucidated.

Phytic acid (IP6) is a natural antioxidant widely present in cereals and legumes (Silva and Bracarense, 2016). Several studies have demonstrated the preventive and therapeutic effects of IP6 in diseases associated to mineral and endocrine disturbances, chronic inflammation, and cancer development (Onomi et al., 2004; Kapral et al., 2012; Fuster et al., 2017). Specifically in models of intestinal inflammation and cancer, IP6 showed favorable

effects decreasing the aberrant crypts formation, increasing cell viability, downregulating pro-inflammatory cytokines and chemokines and decreasing Cox-2 expression (Cholewa et al., 2008; Saad et al., 2013; Kapral et al., 2015).

Swine is one of the most sensitive species to toxic effects of FB1 and DON (Bracarense et al., 2012). Furthermore, the similarity with human intestinal physiology and immune system as well as with the absorption of IP6 (Schlemmer et al., 2001) makes pigs an ideal experimental model to study the toxic effects induced by mycotoxins and strategies to mitigate the toxicity using IP6. In addition, most studies focusing on the effects of FB1 and DON on the oxidative stress were performed *in vitro* or using laboratory animals and chickens (Silva et al., 2018).

In previous *in vitro* (Pacheco et al., 2012) and *ex vivo* (Silva et al., 2014) studies we have showed that IP6 exposure decreased the intestinal lesions induced by FB1 and DON. Nevertheless, the interaction of IP6 on the oxidative stress response produced by mycotoxins in the intestine is not clear. The aim of the present study was to investigate the action of IP6 on the toxic effects induced by FB1 and DON alone and in association on the intestine focusing on the oxidative stress response, morphology, goblet cells density, cell proliferation and apoptosis, E-cadherin and cox-2 expression using jejunal explants of pigs.

## Material and Methods

*Animals and reagents (FB1, DON and phytic acid)*

Five 24-days-old crossbred (Landrace x Large White X Duroc) piglets (7.9 kg ± 0.72) were used in the present study. All animal experimental procedures were performed in accordance with the institutional animal care and use committee (CEUA/UEL/Brazil-process n° 4173.2014.05). The purified DON (MW: 296.32; Sigma–Aldrich, St. Louis, MO, USA) and FB1 (MW: 721.83; Cayman Chemical Company, Michigan, USA) were dissolved in

ultrapure water at final concentration of 10 µM for DON and 70 µM for FB1 and stored at 4 °C. The concentrations of FB1 (70 µM) and DON (10µM) used were described in previous studies (Lucioli et a., 2013; Silva et al., 2014) and are equivalent to 3 mg/ kg feed and 50.5 mg/kg feed, respectively.

The phytic acid salt (MW: 819; Sigma–Aldrich, St. Louis, MO, USA) was dissolved in distilled water in a concentration of 15 mM and the pH adjusted to 7.2. Posteriorly, the solution was stored at -20 °C before dilution in explant culture media. The IP6 concentration of 5 mM used was chosen according preview study (Silva et al., 2014).

*Ex vivo experimental model*

The piglets were euthanized (acepromazine 1%, sodium pentobarbital 40 mg/Kg and KCl 15%), subsequently, the jejunum was excised, and samples 5 cm in length were collected, washed with PBS pH 7,2 solution and opened longitudinally. From each animal, six explants (replicates) were collected to each treatment using a punch 8mm in diameter. The total number of 48 explants were collected from each piglet and laid in 6 well plates (three explants/well) with 3 mL of the following treatments: control (A, B, C and D) - only culture media (DMEM; Gibco-BRL Life Technologies, Carlsbad, CA) plus penicillin/streptomycin (1.25 µL/mL-Gibco-BRL Life Technologies, Carlsbad, CA), gentamicin (10 µL/mL-Novafarma, São Paulo, SP, Brazil), fetal bovine serum (100 µL/mL, Invitrogen, São Paulo, SP, Brazil) and L-glutamine (0.4 µL/mL- Sigma Aldrich, St. Louis, MO, USA); and culture media with IP6 5 mM (E, F, G and H). The explants were incubated at 37 °C using orbital shaking. After one hour, DON (10 µM) was added in the B, D, F and H treatments, and FB1 (70 µM) in the C, D, G and H treatments. The explants returned to incubation under orbital shaking for more three hours. After the incubation period, three explants of each treatment were fixed in 10% neutral buffered formalin solution, dehydrated in alcohols and embedded in paraffin for the histological and immunohistochemical evaluation. Three explants were

immediately frozen in liquid nitrogen and posteriorly stored at -80°C to the assessment of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) levels.

*Histological and immunohistochemical assessment*

Sections of 3µm were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation. An intestinal histological score previously described (Silva et al., 2014) was used to compare the morphological changes and lesions between the treatments. The frequency of each morphological change and lesion was evaluated as previously described (Silva et al., 2014). The following morphological and lesional criteria were included in the score: flattening of enterocytes, villi atrophy and fusion, interstitial edema, lymphatic vessel dilation, loss of apical enterocytes, cell vacuolation and necrotic debris. The lesional score was calculated by taking into the extent of each lesion (according to intensity or frequency observed, scored from 0 to 2; 0-diffuse, 1-moderate and 2-absent).

The villi height was measured randomly on 10 villi using an image analysis system (MOTIC Image Plus Motic Instruments, Richmond, Canada) at 100x magnification. Sections of the jejunum samples were submitted to Periodic Acid-Schiff (PAS) staining to evaluate the goblet cells density. Positive stained goblet cells were counted randomly in 10 villi per explant and their respective bilateral crypts, at 200x magnification.

Sections of the same explants used to histopathological examination were submitted to immunohistochemical assay. The evaluation of apoptosis, cell proliferation, cell junction expression and Cox-2 expression were performed using antibodies against cleaved caspase-3 (Ccasp3) (anti-Asp 175, 1:200 dilution, Cell Signaling Technology, Beverly, MA, USA), Ki-67 (anti-7B11, 1:50 dilution, Zymed, Waltham, MA, USA), E-cadherin (anti-4A2C7, 1:50,

Zymed, Waltham, MA, USA) and Cox-2 (anti-CX-294, 1:100 dilution, Dako, Santa Clara, CA, USA), respectively. The protocols, positive and negative controls were used according to the manufacturer's instructions.

The positive immunoexpression of Ccasp3 and Ki-67 was estimated by counting strongly positive immunostaining of the cytoplasm (Ccasp3) and nucleus (Ki-67) in five random fields in the crypt region/explant at 400x magnification. The expression of E-cadherin was estimated by the evaluation of five fields in the enterocytes of villi region at 200x magnification. The staining was considered positive when homogeneous and strong basolateral membrane staining of the enterocytes was observed. The expression of Cox-2 was estimated by evaluating five fields in the crypt region at 200x magnification. Fields were considered positive when 50% or more of the cells were positively immunostained. The total number of fields evaluated in the three explants per treatment/animal were 15.

*GSH levels measurement*

GSH levels were determined spectrophotometrically by an adapted method described by Sedlack and Lindsay (1968). The frozen intestinal samples were homogenized using Tissue Tearor (Bjospec, São Paulo, SP, Brazil) in cold EDTA buffer (40.02 M). The homogenate was treated with 50% trichloroacetic acid (50% w/v) and centrifuged (1500*g* for 15min), and the supernatant (1 mL) was mixed with 2 mL of a solution 0.4M Tris-HCl, pH 8.9. The samples were vortex-mixed and 10mM dithiobisnitrobenzoic acid was added followed by vortex-mixing. Posteriorly, the samples were allowed to stand for 5 minutes before being read at 412 nm (Multiskan GO Microplate Spectrophotometer, ThermoScientific, Vantaa, Finland). A standard curve was prepared using different concentrations of GSH, in addition to the other reagents mentioned before. Results were presented as nmol GSH/mg of jejunum.

*Lipid peroxidation measurement (TBARS)*

Lipid peroxidation in the jejunum explants was assessed by determining TBARS levels using an adapted method previously described by Guedes et al. (2006). For this assay, trichloroacetic acid (10%) was added to the homogenate to precipitate proteins followed by centrifugation (1000*g*, 3min, 4∘C). The protein-free supernatant was separated and mixed to thiobarbituric acid (0.67%). The mixture was kept in water bath (15 min, 100∘C). Malondialdehyde (MDA), an intermediate product of lipid peroxidation, was determined by the difference between absorbances at 535 and 572 nm using a microplate spectrophotometer reader. The results were presented as TBARS (nmol MDA/mg of jejunum).

*ABTS and FRAP assays*

The ability of intestinal explants in the different treatments to resist oxidative damage was determinated by its free radical scavenging (ABTS assay) and ferric reducing (FRAP assay) properties. The frozen jejunal samples collected were homogenized in ice-cold 1.15% KCl buffer solution. Samples were centrifuged (200g for 10min at 4∘C) and the supernants were used in both assays (ABTS and FRAP) according to Katalinic et al. (2005). The diluted ABTS solution (200 μL) was mixed with 10 μL of sample in each well. After 6 minutes of incubation at 25°C, the absorbance was measured at 730 nm (Multiskan GO Microplate Spectrophotometer, ThermoScientific, Vantaa, Finland). For FRAP assay, the supernatants (10 μL) were mixed with the prepared FRAP reagent (150 μL). The reaction mixture was incubated at 37°C for 30 minutes, and the absorbance was measured at 595 nm. The results of ABTS and FRAP assays were equated using a standard Trolox curve (0.02–20 nmol). Considering these are Trolox equivalent antioxidant capacity (TEAC) assays, results were presented as $\mu$mol Trolox equivalent/mg of jejunum.

*Statistical analysis*

The means of the lesional score, intestinal morphometry, number of goblet cells, positive immunostaining cells for Ki-67 and Ccasp3, positive fields for E-cadherin and Cox-2,

and the mean levels of GSH, TBARS, ABTS and FRAP assays were used for statistical analysis. The data are presented as the means with their standard errors and were analyzed using the free software Action 2.3 (Campinas, SP, Brazil). One-way analysis of variance (ANOVA) followed by a multiple comparison procedure (Tukey's test) was used for means statistical analysis of the lesional score, intestinal morphometry, goblet cells, Ki-67, Ccasp3, E-cadherin and Cox-2. The Duncan's test was used to compare the results of GSH, TBARS, ABTS and FRAP assays. P values of $\leq 0.05$ were considered significant.

## Results

### *Morphology assessment*

After 3 hours of mycotoxins exposure alone or in association, the explants exhibited moderate to severe jejunal lesions mainly in DON plus FB1 treatment (Fig. 1A). The main histological changes observed included multifocal to diffuse cytoplasmic vacuolation and flattening of enterocytes, atrophy and villi fusion, lack of apical epithelium and necrotic debris (Fig.1E-G). Histological scores decreased significantly in the explants exposed to DON (30.2%), FB1 (23%) and DON plus FB1 (38%) compared with the control group. The jejunum exposed to DON presented a significant decrease in the histological score of 9.4% compared to FB1 alone ($p \leq 0.05$) and the treatment DON plus FB1 showed a significant decrease of 23.3% compared to FB1 ($p \leq 0.05$).

Alternatively, when explants were pre-treated with IP6, the histological scores exhibited a significant increase when compared with explants exposed to mycotoxins alone or associated (Fig. 1A). Explants submitted to IP6 alone presented a significant improvement ($p \leq 0.05$) of 24.2% in the morphological score when compared to the control (Fig. 1A, C-D). In addition, explants exposed to IP6 plus mycotoxins exhibited an increase of 42.7% (DON+IP6), 28.6% (FB1+IP6) and 40.4% (DON+FB1+IP6) compared to the respective

treatments with the mycotoxins alone ($p \leq 0.05$). The main histopathological lesions observed in the explants submitted to IP6 pre-treatment were cytoplasmic vacuolation and interstitial edema. The jejunal explants submitted to DON plus IP6 and FB1 plus IP6 showed a lesional score similar to the control treatment ($p \leq 0.05$) (Fig. 1A).

Jejunal explants exposed to mycotoxins exhibited a significant decrease ($p \leq 0.05$) in villi height compared with the control (DON- 40.4%; FB1- 30% and DON plus FB1- 45.4%). However, explants exposed to IP6 and treated with mycotoxins exhibited a significant increase ($p \leq 0.05$) of 54.6% compared to DON alone, 33% compared to FB1alone and 47% compared to DON plus FB1 (Fig. 1B). The jejunal explants exposed to mycotoxins plus IP6 showed a villi height similar to the control explants ($p \leq 0.05$) (Fig.1B, I-K).

The number of goblet cells in the villi of explants exposed to DON alone and DON plus FB1 decreased significantly ($p \leq 0.05$), when compared to control (35.6% and 35%, respectively) (Fig. 2). In the crypt region, the number of goblet cells decreased in DON (34.4%) and DON plus FB1 (39%) treatments ($p \leq 0.05$). Explants exposed to DON+IP6 and DON+FB1+IP6 showed a significant increase ($p \leq 0.05$) in goblet cells density in the crypt region compared to DON alone (40.7%) and DON plus FB1 (53%).

The morphological evaluation showed that the pre-treatment with IP6 in jejunal explants exposed to mycotoxins resulted in a improve of histological score, villi height and goblet cells density similar to observed in the control treatment ($p > 0.05$) (Fig.1-2).

*Caspase-3,Ki-67, E-cadherin and Cox-2 expression*

Casp-3 expression, wich indicates cell apoptosis, decreased 37.3% in the jejunal explants exposed to IP6 compared to the control treatment ($p \leq 0.05$). A significant increase in Casp-3 expression was observed in explants exposed to DON (95.5%) and DON plus FB1 (135.5%) when compared to the control group ($p \leq 0.05$). In addition, an increase of 49.6% in cell apoptosis ($p \leq 0.05$) was verified in DON plus FB1 group compared to FB1 alone.

Nonetheless, a significant decrease of cell apoptosis was observed in DON plus IP6 (46.8%), FB1 plus IP6 (39%) and DON/FB1 plus IP6 (46.8%) groups compared to the respective treatments with the mycotoxins alone ($p \leq 0.05$) (Fig.3A-D).

Explants exposed to IP6 exhibited a decrease of 32.5% ($p \leq 0.05$) in ki-67 expression compared to control samples. Meanwhile, an increase in cell proliferation ($p \leq 0.05$) was observed in explants exposed to DON (28.4%) and DON plus FB1 (40%) when compared to control explants. Jejunal explants submitted to DON plus FB1 treatment, showed a significant increase ($p \leq 0.05$) of 24.7% in the cell proliferation compared to FB1 alone. The presence of IP6 induced a significant decrease ($p \leq 0.05$) in the cell proliferation of jejunal explants exposed to DON (30.3%) and DON plus FB1 (29.6%) compared to explants exposed only to DON and DON plus FB1 (Fig. 3E-H).

In general, exposure to DON alone or associated induced a significant reduction in E-cadherin expression (25.8% and 31%, respectively compared to the control; 20.3% and 26%, respectively compared to the FB1 treatment). However, when explants were pre-incubated with IP6 and subsequently exposed to the mycotoxins, an increase in E-cadherin immunostaining was observed compared to explants exposed to DON (28%), FB1 (15%) and DON plus FB1 (35%) ($p \leq 0.05$) alone (Fig. 4A-D).

The immunoexpression of Cox-2 (parameter used to evaluate the inflammatory response) showed a decrease of 68% in the jejunum treated with IP6 compared with the control samples. In spite of this, explants submitted to the mycotoxins showed a significant increase in Cox-2 expression ($p \leq 0.05$) (150% for DON, 109% for FB1 and 113% for DON plus FB1 compared to the control). The presence of IP6 induced a significant decrease ($p \leq 0.05$) of 51%, 50% and 45.2% in explantes exposed to DON, FB1 and DON plus and FB1, respectively (Fig. 4E-H).

Overall, the addition of IP6 in the culture medium of explants exposed to mycotoxins results in an expression of Casp-3, Ki-67, E-cadherin and Cox-2 similar to control explants indicating a beneficial effect of this product in reducing the toxic effects

*Oxidative stress evaluation*

Jejunal exposure to DON and FB1 induced a significant increase in TBARs levels (62.8% and 54.2%, respectively), while exposure to IP6 resulted in decreased levels when compared to control explants (40%). On the other hand, explants exposed to IP6 and mycotoxins alone or combined showed decreased levels of TBARs (Table 2) compared to explants exposed to mycotoxins ($p \leq 0.05$).

The capacity to respond to oxidative stress was evaluated through GSH, FRAP and ABTS levels. Jejunal explants submitted to mycotoxins treatments showed a significant decrease in endogenous GSH levels compared to the control (DON 62%; FB1 51.8%; DON plus FB1 46.4%) (Table 2). The presence of IP6 induced an increase in GSH levels of 131% and 105.5% compared to DON and FB1 alone; in the explants exposed to DON plus FB1, the IP6 increase was 65.1% in the GSH content.

Explants exposed to mycotoxins presented a significant decrease in the ABTS scavenging ability (DON 33.6%; FB1 35.5%; DON plus FB1 31.1%) compared to the control explants, while pre-exposure to IP6 promoted a significant increase in ABTS capacity in explants exposed to DON (67.9%), FB1 (107%) and DON plus FB1 (105.4%) (Table 2). The ferric reducing ability showed a significant increase in explants exposed to IP6+FB1 (20.6%) and IP6+DON+FB1 (21.4%) compared to explants exposed to these same mycotoxins (Table 2). The previous exposition of jejunal explants to IP6 inhibited the toxic effects of mycotoxins on the lipid peroxidation and the cellular antioxidant capacity .

**Discussion**

This study investigated the protective effect of IP6 on the jejunum explants of swine exposed to FB1 and DON. Intestinal morphological changes induced by these mycotoxins alone (Silva et al., 2014) or in association (Bracarense et al., 2012) have been previously established and were similar to the observed in the present study. The intestinal epithelial cells (IEC) changes observed after explants exposure to mycotoxins such as cytoplasmatic vacuolation, fattening, necrosis and loss of microvilli were probably induced by an increase in cytoplasmic and mitochondrial permeability and may be associated to ROS generation, resulting in lipid peroxidation as observed *in vitro* (Li et al., 2014; Domijan et al., 2015) and *in vivo* (Osselaere et al., 2013; Abbes et al., 2016).

The reduction in the number of goblet cells and villi height evidenced mainly in DON and FB1 plus DON groups are may linked to increased levels of apoptosis. This association is supported by an increased caspase-3 immunostaining in these groups. DON induce apoptosis by direct lesion of mitochondria (ribotoxic stress) and can also activate the extrinsic pathway through TNF-α as a consequence of intestinal inflammation (Li et al., 2014). Considering the concentration of DON used and the short period of incubation, the elevated apoptosis index observed may be associated with the activation of the intrinsic apoptosis pathway linked to the oxidative stress and changes in mitochondrial membrane potential (MMP), deregulation of Bcl-2/Bax expression, release of cytochrome C and consequent activation of caspase-3. In addition, it has been established that DON can modulate the cell proliferation and apoptosis through activation of MAPKs, mainly ERK1/2, JNK 1/2 and p38 (Yang et al., 2000; Pestka, 2003; Lucioli et al., 2013). This modulation of cell proliferation was observed in the present study. The explants exposed to DON alone and FB1 plus DON showed discrete elevation of cell proliferation in the crypt region and it likely occurred as a tissue response to IEC lesions and high apoptosis level induced by DON.

Alterations in cell junction proteins (Basso et al., 2013), transepithelial electrical resistance (TEER) and paracellular permeability (Pinton et al., 2010) were associated with DON intestinal toxicity. In the present study, explants exposed to DON and FB1 plus DON showed a decreased E-cadherin expression compared with the control treatment. This reduction is possibly associated to morphological changes observed in the IEC and the oxidative stress, since the ROS generation induced by DON is associated to inhibition of protein synthesis (Strasser et al., 2013) and activation of mitogen activated protein kinases (MAPKs) (Pinton et al., 2010).

Besides deregulation of cell proliferation and apoptosis, the oxidative stress can induce an overexpression of Cox-2 (Reuter et al., 2010). Cox-2 is an enzyme involved in the metabolism of arachidonic acid which is strongly induced by p38 MAPK activation and proinflammatory stimuli such as the expression of TNF-α, IL-1, IL-6 and IL-8 (Cuenda and Rousseau, 2007). Studies have been demonstrated that FB1 and DON induced upregulation of pro-inflammatory cytokines in pigs (Bracarense et al., 2012; Grenier et a., 2012) and increase the expression of Cox-2 (Silva et al., 2014). In the present study, the expression of Cox-2 increased significantly in all mycotoxins treatments demonstrating its role as biomarker of intestinal oxidative stress and inflammatory response in this model. Evaluation of oxidative stress involves free radicals generation (resulting in lipid peroxidation) and antioxidant capacity. In this study, mycotoxins alone or associated affected both mechanisms. An increase in lipid peroxidation was observed mainly in the monocontaminated treatments (~1.5 fold). On the other hand, the GSH levels decreased after mycotoxin exposure (~2.2 fold) and also the ABTS and FRAP levels (~1.5 and 1.1 fold, respectively).

Reduction in superoxide dismutase (SOD) , catalase (CAT), glutathione peroxidase (GPx) and GSH levels were reported in *in vitro* and *in vivo* studies after DON (Hou et al., 2013; Strasser et al., 2013; Zbynovska et al., 2013) and FB1 exposure (Domijan et al., 2015;

Abbes et al., 2016). DON toxicity was associated with ROS generation giving rise to changes in lysossomal membrane, and increase in mitochondrial membrane permeability with consequent deregulation of Bcl-2/Bax genes, releasing of cytochrome C and activation of caspase-3 (Li et al.,2014; Sun et al., 2015). Similarly to DON, the oxidative stress induced by FB1 results in mitochondrial lesion and activation of caspase-3 (Abbes et al., 2016; Wang et al., 2016; Mary et al., 2017). The effects of DON on oxidative stress parameters were more evident than FB1, however, no significant difference was verified. We hypothesize that explants exposed to FB1 present similar oxidative stress to DON because the former presented less epithelial cells in apoptosis executor phase (activation of caspase-3) compared to DON treatment. Therefore, the real potential of oxidative stress induced by DON was masked, since several studies have demonstrated that DON rapidly induces a ribotoxic stress and ROS generation mainly affecting cells with high cell division index, such as intestinal epithelial cells (Pestka and Smolinski, 2005). The oxidative stress induced by FB1 occurs indirectely via the intracellular accumulation of sphingolipids (Grenier et a., 2012), a toxic mechanism with slower progression compared to DON toxic effect at ribosomal level.

The presence of IP6 induced a protective effect on all histological parameters evaluated. This effect could be observed by the increased histological score, villi height and goblet cell density compared to jejunal explants exposed to the mycotoxins alone and associated treatments. The beneficial effects of IP6 are associated with its antioxidant capacity, mainly its ability to inhibit the Fenton reaction and formation of hydroxyl radicals (Graf and Eaton, 1990). The evaluation of cell proliferation, apoptosis and E-cadherin expression suggest that IP6 modulate the toxic effects through a decrease in ROS generation and consequently less changes in cell permeability and MAPKs activation resulting in the maintenance of protein synthesis. The elevated reduction of apoptosis by IP6 observed in all mycotoxins treatment demonstrated their potent antioxidant effect, since ROS generation is

directly linked to apoptosis activation. Similar modulation of cell viability was observed in bowel inflammatory studies (Challa et al., 1997; Jenab and Thompson, 2000; Kapral et al., 2015). IP6 promoted a significant reduction in the Cox-2 expression compared to explants control and exposed to all mycotoxins treatments. This reduction in the expression of Cox-2 observed in the present study can be associated to the ability of IP6 to inhibit the ROS production, lipid peroxidation and decrease the inflammatory stimuli (Norazalina et al., 2010). Moreover, studies demonstrated that IP6 reduced the expression of Cox-2 by inhibition of p38 MAPK and conversion of arachidonic acid into prostaglandins, and suppression of β-catenin activity (Norazalina et al., 2010; Shafie et al., 2013; Kapral et al., 2015).

In the evaluation of oxidative stress, the presence of IP6 decreased in 66.6% and 48.8% the MDA levels in the explants submitted to DON and FB1 treatments, respectively. The presence of IP6 reduced 48.8% of MDA content compared with mycotoxins combined treatment. Furthermore, the IP6 promoted a significant increase in the GSH level and antioxidant capacity in jejunal explants exposed to mycotoxins. Nevertheless, IP6 ability to protect the cell against oxidative stress was associated wth inhibition of ROS generation, increase of GSH level, CAT, GPx and SOD content, and decrease of lipid peroxidation (MDA) in hepatocarcinogenesis studies in rats (Lee et al., 2005; Abdel-Hamid et al., 2007; Foster et al., 2017), however, data about the effect of IP6 on oxidative stress at intestinal level are scarce. The present results demonstrate that the protective effect of IP6 on the intestinal oxidative stress is associated with its capacity in decrease the lipid peroxidation and increase the antioxidant capacity of the tissue.

The morphological changes and oxidative stress response were more evident in explants exposed to DON and DON plus FB1 treatments and are associated to rapid and direct induction of oxidative stress by DON compared to FB1-induced injury. Besides FB1 and

DON co-contamination of food and feed has been reported worldwide (Binder et al., 2007) with the possibility of synergistic toxic effects (Bracarense et al., 2012), the histopathological, immunohistochemistry and oxidative stress parameters evaluated showed no synergistic effect. The synergistic, additive, less-than additive or antagonist effects observed in mycotoxins multi-contaminations depend of concentration, period of exposition, experimental model and specie susceptibility (Grenier; Oswald, 2011).

**Conclusion**

The mycotoxins DON and FB1, alone or in association induced changes in morphology, protein junction, cell proliferation, and apoptosis on swine jejunum. These toxic effects are associated to an oxidative stress response, including lipid peroxidation and antioxidant capacity. However, more studies are necessary to elucidate the mechanism and pathways of oxidative stress induced by the mycotoxins at intestinal level. The IP6 exerts benefits effects upon the jejunum, modulating the changes induced by the mycotoxins and protecting the cells against the oxidative stress. In this context, the IP6 antioxidant additives shown efficient properties in mitigate and prevent the toxic effects of the DON and FB1 on the jejunum explants of pigs.

**Acknowledgements**

This study was supported by a grant from CNPq (474691/2012-8) and Fundação Araucária, Brazil.

**References**

Abbes, S., Ben Salah-Abbes, J., Jebali, R., Younes, R.B., Queslati, R. 2016. Interaction of aflatoxin B1 and fumonisin B1 in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. J. Immunotoxicol. 13, 46-54.

Abdel-Hamid, N.M., Faddah, L.M., Al-Rehany, M.A., Ali, A.H., Bakeet, A.A. 2007 . New role of antinutritional factors, phytic acid and catechin in the treatment of CCl4 intoxication. Ann. Hepatol. 6, 262-266.

Adhikari, M., Negi, B., Kaushik, N., Adhikari, A., Al-Khedhairy, A.A., Kaushik, N.K., Choi, E.H. 2017. T-2 mycotoxin: toxicological effects and decontamination strategies. Oncotarget 8, 33933-33952.

Alshannaq, A., Yu, J.-H. 2017. Occurrence, toxicity, and analysis of the major mycotoxins in food. Int. J. Environ. Res. Public Health. 14, E632.

Basso, K., Gomes, F., Bracarense, A.P. 2013. Deoxynivanelol and fumonisin, alone or in combination, induce changes on intestinal junction complexes and in E-cadherin expression. Toxins (Basel). 5, 2341-2352.

Bracarense, A.P.F.R.L., Lucioli, J., Grenier, B., Pacheco, G.D., Molls, W.-D., Schtzmayr, G., Oswald, I.P. 2012. Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. Br. J. Nutr. 107, 1776–1786.

Challa, A., Rao, D.R., Reddy, B. 1997. Interactive suppression of aberrant crypt foci induced by azoxymethene in rat coln by phytic acid and green tea. Carcinogenesis. 18, 2023-2026.

Cholewa, K., Parfiniewicz, B., Bednarek, I., Swiatkowska, L., Jezienicka, E., Kierot, J., Weglarz, L. 2008. The influence of phytic acid on TNF-α and its receptors genes expression in colon cancer caco-2 cells. Acta. Polon. Pharm-Drug. Res. 65, 75-79.

Cuenda, A., Rousseau, S. 2007. p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim. Biophys. Acta. 1773, 1358-1375.

Domijan, A.M., Gajski, G., Jovanovic, I.N., Geric, M., Garaj-Vrhovac, V. 2015. *In vitro* genotoxicity of mycotoxin ochratoxin A and fumonisin B (1) could be prevented by sodium copper chlorophylin-implication to their genotoxic mechanism. Food. Chem. 170, 455-462.

Escrivá, L., Font, G., Manyes, L., 2015. In vivo toxicity studies of Fusarium mycotoxins in the last decade: a review. Food Chem. Toxicol. 78, 185-206.

Foster, S.R., Dilworth, L.L., Thompson, R.K., Alexander-Lindo, R.L., Omoruyi, F.O. 2017. Effects of combined inositol hexakisphosphate and inositol supplement on antioxidant activity and metabolic enzymes in the liver of streptozotocin-induced type 2 diabetic rats. Chem. Biol. Interact. 275, 108-115.

Fuster, J.M.B., Cortes, S.P., Bestard, J.P., Freixedas, F. 2017. Plant phosphates, phytate and pathological calcifications in chronic kidney disease. Nefrologia. 37 20-28.

Graf, E., Eaton, J.W. 1990. Antioxidant functions of phytic acid. Free Radic. Biol. Med. 8, 61-69.

Grenier, B., Oswald, I.P. 2011. Mycotoxin co-contamination of foods and feeds: meta-analysis of publications describing toxicological interactions. World Mycotoxin J. 4, 285–313.

Grenier, B., Bracarense, A.P.F.L., Schwartz, H.E., Trumel, C., Cossalter, A.M., Schatzmayr, G., Oswald, I.P. 2012. The low intestinal and hepatic toxicity of hydrolyzed fumonisin B1 correlates with its inability to alter the metabolism of sphingolipids. Biochem. Pharmacol. 83, 1465-1473.
Guedes, R.P., Dal Bosco, L., Teixeira, C.M., Llesuy, S., Belló-Klein, A., Ribeiro, M.F., Partata, W.A., 2006. Neuropathic pain modifies antioxidante activity in rat spinal cord. Neurochem. Res. 31, 603-609.

Hou, Y.J., Zhao, Y.Y., Xiong, B., Cui, X.S., Kim, N.H., Xu, Y.X., Sun, S.C. 2013. Mycotoxin-containing diet causes oxidative stress in the mouse. PLoS ONE. 8, e60374.

Jenab, P.M., Thompson, L.U. 2000. Phytic acid in wheat bran affects colon morphology, cell proliferation and apopstosis. Carcinogenesis. 21, 1547-1552.

Kapral, M., Wawszyk, J., Jurzak, M., Hollek, A., Weglarz, L. 2012. The effect of inositol hexaphosphato on the expression of selected metalloproteinase and their tissue inhibitors in Il-1β-stimulated colon. Int. J. Colorectal Dis. 27, 1419-1428.

Kara, M., Wawszczyk, J., Sośnicki, S., Węglarz, L. 2015. Down-regulation of inducible nitric oxide synthase expression by inositol hexaphosphate in human colon cancer cells. Acta Pol. Pharm. 72, 705-711.

Katalinic, V., Modun, D., Music, I., Boban, M., 2005. Gender differences in antioxidant capacity of rat tissues determined by 2,2'-azinobis (3-ethylbenzothialoine 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 140, 47-52.

Lee, H.J., Lee, S.A., Choi, H. 2005. Dietary administration of inositol and/or inositol-6-phosphate prevents chemically-induced rat hepatocarcinogenesis. Asian Pac. J. Cancer Prev. 6, 41-47.

Li, D., Ye, Y., Lin, S., Deng, L., Fan, X., Zhang, Y., Deng, X., Li, Y., Haikuo, M.H.Y. 2014. Evaluation of deoxynivalenol-induced toxic effects on DF-1 cells in vitro: cellcycle arrest, oxidative stress, and apoptosis. Environ. Toxicol. Pharmacol. 37,141–149.

Lucioli, J., Pinton, P., Patrick, C., Laffitte, J., Grosjean, F., Kolf-Clauw, M., Oswald, I.P., Bracarense, A.P.F.R.L. 2013. The food contaminant deoxynivlanol activates the mitogen activated protein kinases in the intestine: interest of *ex vivo* models as an alternative to *in vivo* experiments. Toxicon. 66, 31-36.

Mary, V.S., Theumer, M.G., Otaiza, S.N., Velez, P.A., Rubinstein, H.R., Theumer, M.G. 2017. The aflatoxina B1-fumonisin B1 toxicity in BRL-3A Hepatocytes is associated to induction of cytochrome P450 activity and arachidonic acid metabolism. Environ. Toxicol. 32, 1711-1724.

Norazalina, S., Norhaizan, M.E., Hairuszah, I., Norashareena, M.S. 2010. Anticarcinogeninc efficacy of Phytic acid extracted from rice bran on azoxymethane-induced colon carcinogenesis in rats. Exp. Toxicol. Pathol. 62, 259-268.

Onomi, S., Okazak, Y., Katayama, T. 2004. Effect of dietary level of phytic acid on heptic and serum lipid status in rats fed a high-sucrose diet. Biosc. Biotecnol. Biochem. 68, 1379-1381.

Osselaere, A., Santos, R., Hautekiet, V., Backer, P.D., Chiers, K., Ducatelle, R., Croubels, S. 2013. Deoxynivalenol impairs hepatic and intestinal gene expression of selected oxidative stress, tight junction and inflammation proteins in broiler chickens, but addition of an adsorbing agent shifts the effects to the distal parts of the small intestine. PLoS ONE. 8, e69014.

Pacheco, G.D., Silva, C.A., Pinton, P., Oswald, I.P., Bracarense, A.P., 2012. Phytic acid protects porcine intestinal epithelial cells from deoxynivalenol (DON) cytotoxicity. Exp Toxicol. Pathol. 64, 345-347

Pesta, J.J. 2008. Mechanisms of deoxynivalenol-induced gene expression and apoptosis. Food Addit. Contam. A Chem. Anal Control Exp. Risk Assess. 25, 1123-1140.

Pestka, J.J, Smolinski, A,T. 2005. Deoxynivalenol: toxicology and potential effects on humans. J. Toxicol. Environ. Health B Crit. Rev. 8, 39-69.

Pinton, P., Braicu, C., Nougayred, J.-P., Laffitte, J., Taranu, I., Oswald, I.P. 2010. Deoxynivalenol impairs porcine intestinal barrier function and decreases the protein expression of claudin-4 through a mitogen activated protein kinase-dependent mechanism. J. Nutr. 140, 1956-1962.

Reuter, S., Gupta, S.C., Vhaturvedi, M.M., Agarwal, B.B. 2010. Oxidative stress, inflammation, and cancer: how are they linked? Free Rad. Biol. Med. 49, 1603-1616.

Saad, N., Esa, N.M., Ithnin, H. 2013. Suppression of β-catenin and cyclooxygenase-2 expression and cell proliferation in zoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA). Asian Pac. J. Cancer Prev. 14, 3093-3099.

Schlemmer, U., Jany, K.D., Berk, A., Schulz, E., Rechkemmer, G. 2001. Degradation of phytate in the gut of pigs-pathway of gastrointestinal inositol phosphate hydrolysis and enzyme involved. Arch. Anim. Nutr. 55, 255-280.

Sedlak, J., Lindsay, R. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagente. Anal Biochem. 25, 192-205.

Shafie, N.H., Esa, N.M., Ithnin, H., Akim, A.M., Saad, N., Pandurangan, A.K. 2013. Preventive inositol hxaphosphate extracted from rice bran inhibits colorectal cancer through involvement of Wnt/β-catenin and cox-2 pathways. Biomed. Res. Inter. ID 681027.

Silva, E.O., Bracarense, A.P.F.R.L. 2016. Phytic acid: from antinutritional to multiple protection of organic systems. J. Food Sci. 81, R135-R1362.

Silva, E.O., Bracarense, A.P.F.R.L., Owald, I.P. 2018. Mycotoxins and oxidative stress: where are we? World Mycotoxin J. 11, 113-133.

Silva, E.O., Gerez, J.R., Drape, T.C., Bracarense, A.F.R.L. 2014. Phytic acid decreases deoxynivalenol and fumonisin B1-induced changes on swine jejunal explants. Toxicol. Rep. 1, 284-292.

Strasser, A., Carra, M., Ghareeb, K., Awad, W., Bohm, J. 2013. Protective effects of antioxidants on deoxynivalenol-induced damage in murine lymphoma cells. Mycotoxin Res. 29, 203-208.

Sun, L.H., Lei, M., Zhang, N.Y., Gao, X., Li, C., Krumm, C.S., Qi, D.S. 2015. Individual and combined cytotxic effects of aflotoxin B1, zearalenone, deoxynivalenol and fumonisin B1 on BRL3A rat liver cell. Toxicon. 96, 6-12.

Wang, X., Wu, Q., Wan, D., Liu, Q., Chen, D., Liu, Z., Matinez-Larranaga, M.R., Martinez, M.A., Anadon, A., Yuan, Z. 2016. Fumonisins: oxidative stress-mediated toxicity and metabolism in vivo and in vitro. Arch. Toxicol. 90, 81-101.

Yang, G.-H., Jarvis, B.B., Vhung, Y.-J., Pestka, J.J. 2000. Apoptosis induction by the Satratoxins and other trichothecene mycotoxin: relationship to ERK, p38 MAPK, and SAPK/JNK activation. Toxicol. Appl. Pharmacol. 164, 149-160.

Zbynovska, K., Petruška, P., Capcarova, M. 2013. Effect of deoxynivalenol on some haematological, biochemical and antioxidant parameters of porcine blood in vitro. J. Microbiol. Biotechnol. Food Sci. 2, 1611–1628

Silva, E.O., Bracarense, A.P.F.R.L., Owald, I.P. 2018. Mycotoxins and oxidative stress: where are we? World Mycotoxin J. 11, 113-133.

Silva, E.O., Gerez, J.R., Drape, T.C., Bracarense, A.F.R.L. 2014. Phytic acid decreases deoxynivalenol and fumonisin B1-induced changes on swine jejunal explants. Toxicol. Rep. 1, 284-292.

Strasser, A., Carra, M., Ghareeb, K., Awad, W., Bohm, J. 2013. Protective effects of antioxidants on deoxynivalenol-induced damage in murine lymphoma cells. Mycotoxin Res. 29, 203-208.

Sun, L.H., Lei, M., Zhang, N.Y., Gao, X., Li, C., Krumm, C.S., Qi, D.S. 2015. Individual and combined cytotxic effects of aflotoxin B1, zearalenone, deoxynivalenol and fumonisin B1 on BRL3A rat liver cell. Toxicon. 96, 6-12.

Wang, X., Wu, Q., Wan, D., Liu, Q., Chen, D., Liu, Z., Matinez-Larranaga, M.R., Martinez, M.A., Anadon, A., Yuan, Z. 2016. Fumonisins: oxidative stress-mediated toxicity and metabolism in vivo and in vitro. Arch. Toxicol. 90, 81-101.

Yang, G.-H., Jarvis, B.B., Vhung, Y.-J., Pestka, J.J. 2000. Apoptosis induction by the Satratoxins and other trichothecene mycotoxin: relationship to ERK, p38 MAPK, and SAPK/JNK activation. Toxicol. Appl. Pharmacol. 164, 149-160.

| Treatment | GSH (nmol/ mg of protein) | TBARS ($\Delta$OD $A_{535}$-$A_{532}$/ mg protein) | ABTS (nmol/Trolox Eq/ mg of protein) | FRAP (nmol Trolox Eq/ mg of protein) |
|---|---|---|---|---|
| Control | 21.79±0.49$^{a}$ | 0.35±0.04$^{a}$ | 60.70±6.65$^{a}$ | 60.23±1.68$^{abc}$ |
| IP6 | 23.24±2.39$^{a}$ | 0.21±0.02$^{b}$ | 64.00±8.19$^{ab}$ | 57.14±2.77$^{abc}$ |
| DON | 8.27±2.25$^{b}$ | 0.58±0.05$^{c}$ | 40.26±5.93$^{c}$ | 51.40±2.90$^{c}$ |
| DON+IP6 | 19.17±2.37$^{a}$ | 0.19±0.02$^{b}$ | 67.60±4.19$^{abd}$ | 66.15±2.31$^{abd}$ |
| FB1 | 10.50±3.66$^{b}$ | 0.54±0.03$^{cd}$ | 39.14±8.66$^{c}$ | 58.99±5.25$^{abc}$ |
| FB1+IP6 | 21.56±1.67$^{a}$ | 0.22±0.03$^{b}$ | 81.00±2.64$^{ad}$ | 71.77±1.91$^{d}$ |
| DON+FB1 | 11.67±1.64$^{b}$ | 0.43±0.06$^{ad}$ | 41.83±1.09$^{c}$ | 55.77±5.35$^{bc}$ |
| DON+FB1+IP6 | 19.26±1.52$^{a}$ | 0.23±0.02$^{b}$ | 85.75±5.60$^{d}$ | 67.71±8.86$^{ad}$ |

**Table 1.** Effects of FB1, DON and IP6 on the oxidative stress in jejunal explants of swine.

Mean values with their standard errors (n 5 animals). Duncan's test.
Mean values with unlike superscript letters (column) were significantly different for each test ($p \leq 0.05$).
GSH-reduced glutathione; TBARS- thiobarbituric acid reactive substances; ABTS-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid;
FRAP- ferric reducing antioxidant power

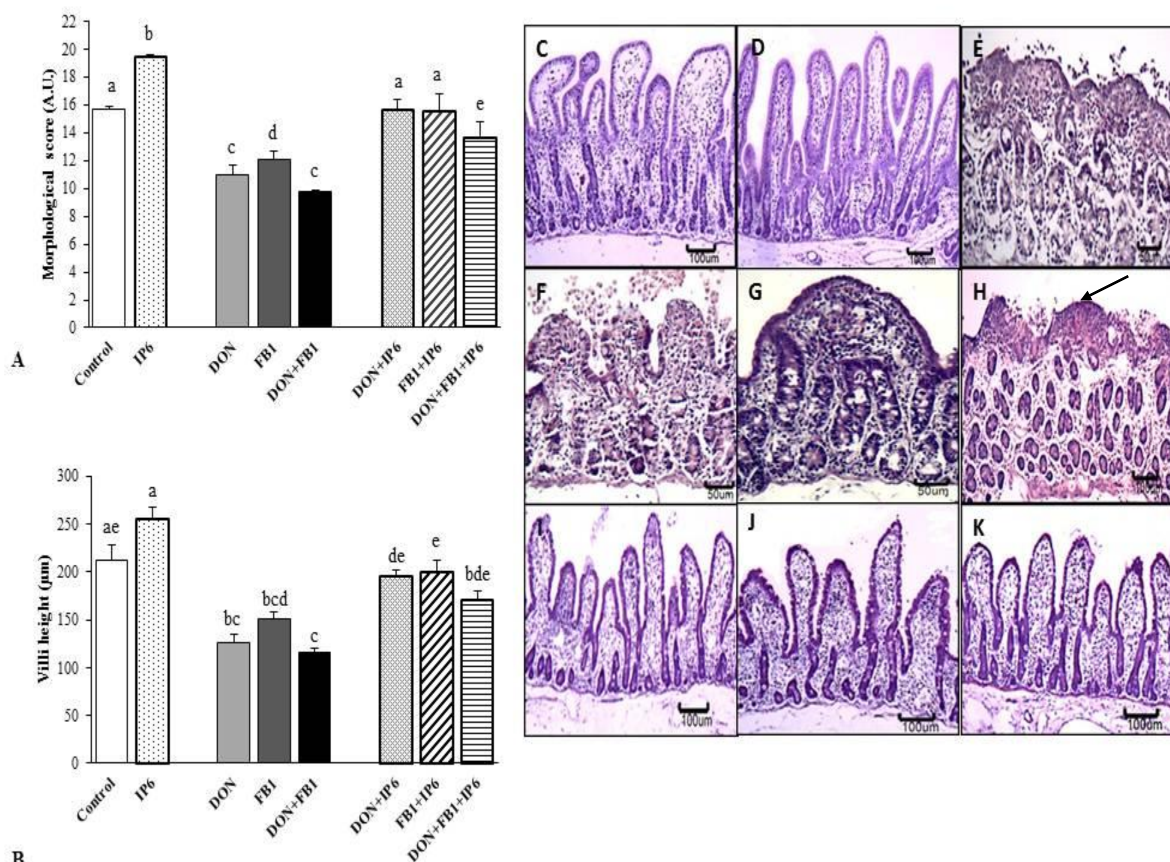



**Figure 1.** Effects of DON, FB1 alone or associated and IP6 on histological morphology in jejunal explants. **A**-Morphological score (AU-Arbitrary Units). **B**-Villi height (µm). Mean values with unlike superscript letters were significantly different ($p \leq 0.05$). **C**-Control treatment. HE, bar 100µm. **D**-IP6 5 mM treatment. HE, bar 100µm. **E**-DON 10 µM alone: severe villi atrophy and fusion, and loss of the apical enterocytes. HE, bar 50µm **F**-FB1 alone: severe loss of apical enterocytes. HE, bar 50µm. **G**-DON 10 µM plus FB1: severe villi fusion. HE, bar 50µm. **H**-DON 10 µM plus FB1: flattened enterocytes (arrow), villi atrophy and loss of enterocytes. HE, bar 100µm. **I**- DON 10 µM + IP6 5 mM: histological aspects similar to the control group. HE, bar 100µm. **J**-FB1+IP6 5 mM: histological aspects similar to the control group. HE, bar 100µm. **K**-DON 10 µM plus FB1 + IP6 5 mM: histological aspects similar to the control group. HE, bar 100µm.

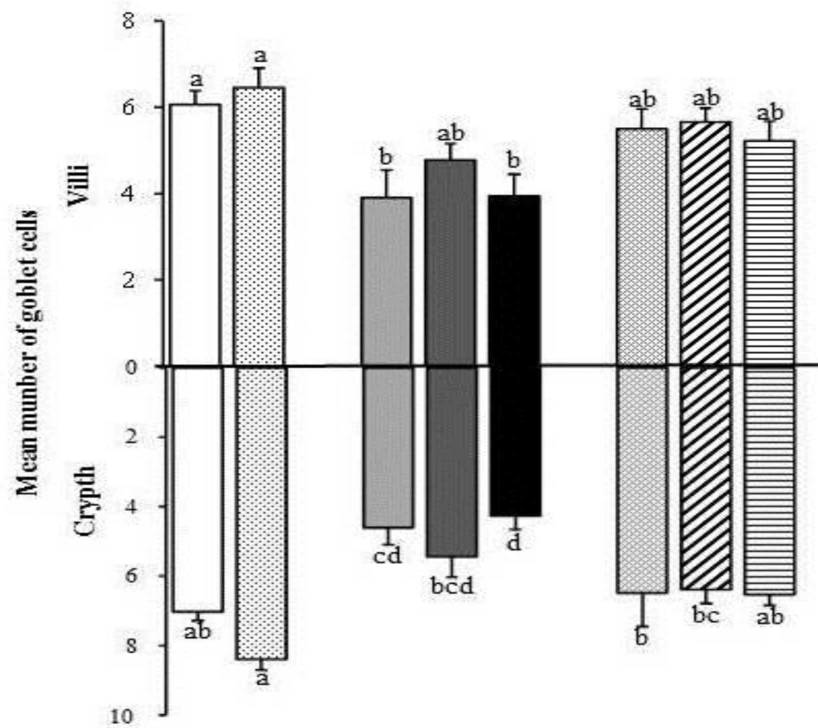



**Figure 2.** Mean goblet cells density on villi and crypt of jejunal explants. Control treatment ; IP6 5 mM ; DON 10 µM ; DON 10 µM + IP6 5 mM ; FB1 70 µM ; FB1 70 µM + IP6 5 mM ; DON 10 µM plus FB1 70 µM ; DON 10 µM plus FB1 70 µM + IP6 5 m. Mean values with unlike superscript letters were significantly different ($p \leq 0.05$).

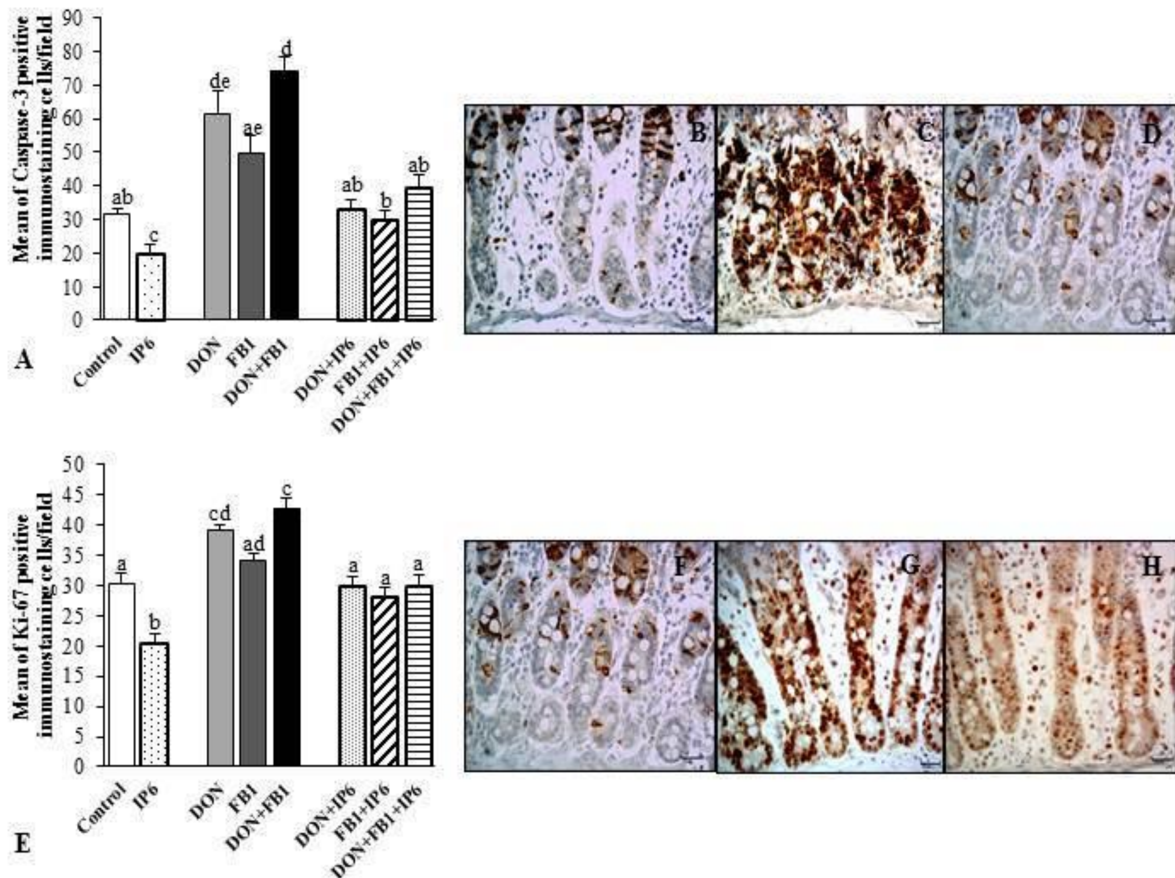



**Figure 3.** Effects of DON, FB1 alone or associated and IP6 on apoptosis (Ccasp-3) and cell proliferation (ki-67) in jejunal explants. **A**- Mean number of Ccasp-3 immunostained cells per field on explants exposed to different treatments. Mean values with unlike superscript letters were significantly different ($p \leq 0.05$). **B**-Control treatment: mild Ccasp-3 cytoplasmic immunostaining in crypt cells. **C**-DON 10 µM plus FB1 70 µM : diffuse Ccasp-3 cytoplasmic immunostaining in crypt cells. **D**-DON 10 µM plus FB1 70 µM + IP6 5 mM: decrease in Ccasp-3 cytoplasmic immunostaining in crypt cells. **E**- Mean number of ki-67 immunostained cells per field on explants exposed to different treatments. Mean values with unlike superscript letters were significantly different ($p \leq 0.05$). **F**-Control treatment: mild ki-67 nuclear immunostaining in crypt cells. **G**-DON 10 µM plus FB1 70 µM : diffuse ki-67 nuclear immunostaining in crypt cells. **H**-DON 10 µM plus FB1 70 µM + IP6 5 mM: decrease in ki-67 nuclear immunostaining in crypt cells. (B-D; F-H: immunoperoxidase method, bar 25µm).

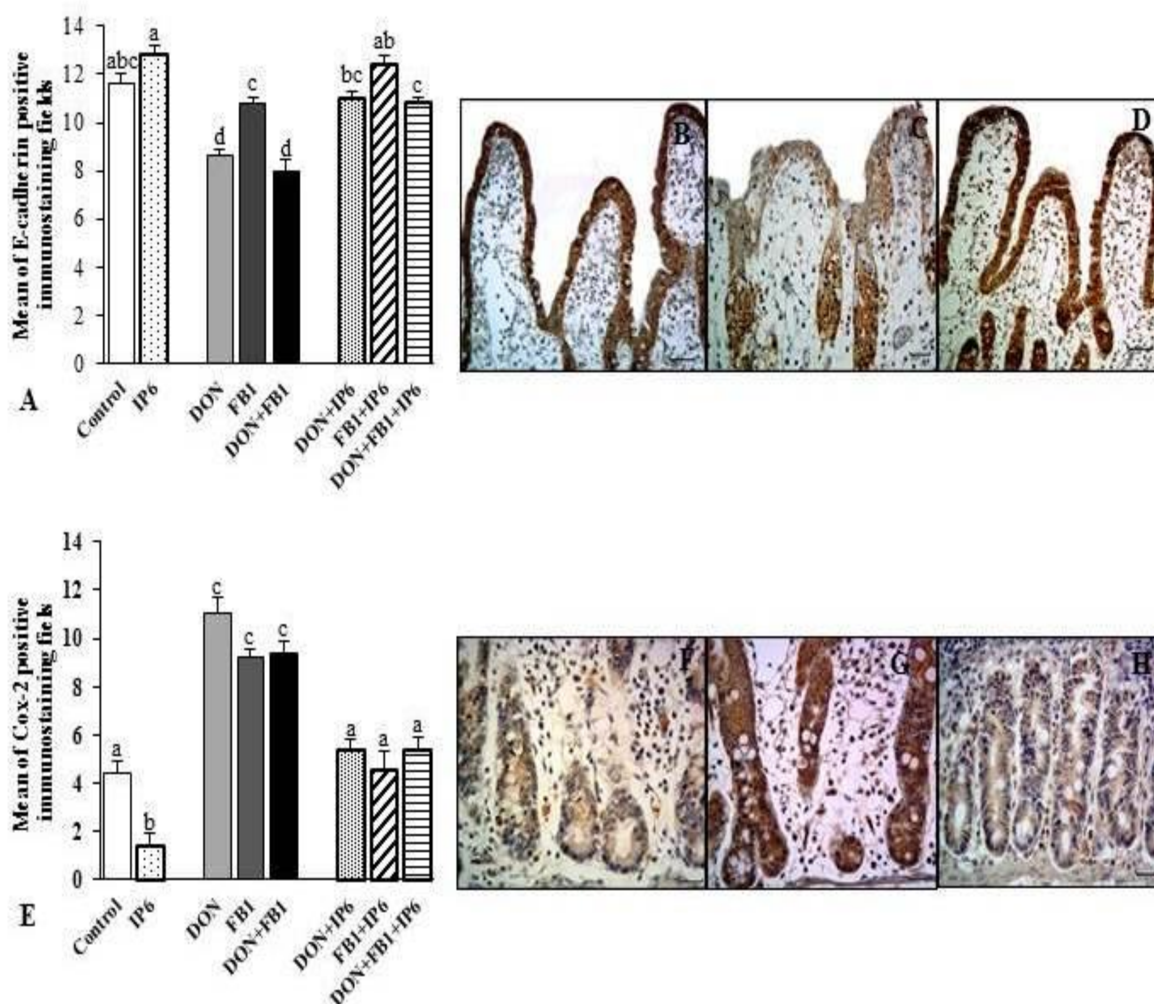



**Figure 4.** Effects of DON, FB1 alone or associated and IP6 on the E-cadherin and Cox-2 expression in jejunal explants. **A**- Mean of E-cadherin positive immunostaining per fields on explants exposed to different treatments. Mean values with unlike superscript letters were significantly different ($p \leq 0.05$). **B**-Control treatment: strong and homogeneous E-cadherin immunostaining in epithelial cells. bar 50µm.**C**-DON 10 µM: mild and non-homogeneous E-cadherin immunostaining in epithelial cells. bar 25µm.**D**-DON 10 µM + IP6 5 mM: strong and homogeneous E-cadherin immunostaining in epithelial cells similar to control treatment. bar 50µm. (B-D: immunoperoxidase method).**E**- Mean of Cox-2 positive immunostaining per fields on explants exposed to different treatments. Mean values with unlike superscript letters were significantly different ($p \leq 0.05$). **F**-Control treatment: mild Cox-2 cytoplasmic immunostaining in crypt cells. **G**-DON 10 µM: diffuse and strong Cox-2 cytoplasmic immunostaining in crypt cells. **H**-DON 10 µM + IP6 5 mM: decrease in Cox-2 cytoplasmic immunostaining in crypt cells similar to control treatment. (B-D: immunoperoxidase method, bar 25µm).

## 5 Artigo 2

# PHYTIC ACID MODULATES THE IMMUNOLOGICAL RESPONSE OF CYTOKINES AND β-DEFENSINS IN PORCINE INTESTINE EXPOSED TO DEOXYNIVALENOL AND FUMONISIN B1[3]


**Abstract**

The worldwide occurrence of mycotoxins in agricultural products represents a risk for human and animal health and is linked to significant economic losses. Therefore, there is a requirement of researches and strategies to mitigate or even eliminate their harmful impacts. This study investigated the effects of phytic acid (IP6) on the immunological response of pro- (IL-1β, IL-6, IL-8, IL-10, IFN-γ, TNF-α) and anti-inflammatory (IL-10) cytokines and β-defensins 1 (pBD-1) and 2 (pBD-2) in porcine jejunal explants exposed to deoxynivalenol (DON) and fumonisin B1 (FB1). The jejunal explants were exposed during 4 h to the following treatments: control, DON (10μM), DON plus IP6 2.5 mM or 5 mM, FB1 (70 μM), FB1 plus IP6 2.5mM or 5 mM. The expression levels of the cytokines were measured by RT-PCR. The explants exposed to DON showed an increase in the expression of IL-1β and IL-8 and a decrease in the levels of IL-6, IFN-γ, IL-10 and pBD-2 ($p<0.05$). The presence of IP6, mainly the higher concentration (5mM), decreased the expression of IL-8 and increased the expression of pBD-1 and 2 compared to DON alone ($p<0.05$). FB1 induced a significant decrease in the levels of most of the pro-inflammatory cytokines, IL-10 and pBD-1, and an increase in IL-1β expression ($p<0.05$). The addition of IP6 5 mM induced significant increase of TNF-α expression compared to FB1 ($p<0.05$). Taken together, the results suggest that IP6 modulates immunological changes induced by DON and FB1 on intestinal mucosa resulting in beneficial effects that contribute to intestinal homeostasis and health.


**Keywords:** DON, FB1, IP6, intestinal immunology, swine.

## Introduction

---
[3] Artigo editado de acordo com as normas de publicação da revista Food and Chemical Toxicology (https://www.journals.elsevier.com/food-and-chemical-toxicology)

The intestine is a complex organ with several functions that serves as a primary barrier against the ingestion of antigens, chemicals, natural toxins and contaminated food (Bouhet; Oswald, 2005). The intestinal epithelial cells play an important role in the intestinal barrier and mucosal immune system by the local production of inflammatory mediators (cytokines and chemokines) and antimicrobial peptides (AMPs) (Bevins et al., 1999; Stadnyk, 2002).

Cytokines are important mediators in the regulation of immune and inflammatory responses (Stadynik, 2002) produced by cells of the immune system (macrophages, lymphocyte, dendritic cells) as well as intestinal epithelial cells (Oswald, 2006). In the intestine, the cytokines IL-6, IL-8, IL-1β, IFN-γ and TNF-α are chemotactic and activators of inflammatory cells, and are produced by normal enterocytes. These cytokines are up-regulated in microbial infections and cellular injury due to chemical and toxic agents inducing an inflammatory response (Jung et al., 1995; Stadynik, 2002). On the other hand, the expression of anti-inflammatory cytokines as IL-4 and IL-10 can protect intestinal tissues from inflammation by the inhibition of inflammatory cells activation, control of the influx of immune cells and regulation of cellular growth and differentiation (Huang et al., 1996; Szkaradkiewicz et al., 2009).

Defensins are the major family of AMPs and can be distinguished in α- and β-defensins (Bevins et al., 1999). The α-defensins are constituents of the primary granules of granulocytes as neutrophils and may be expressed in Paneth cells (Selsteed et al., 1992) and were identified in humans, monkeys and rodents (Yang et al., 2002). The β-defensins are synthesized by epithelial cells lining the respiratory, urogenital and gastrointestinal systems of mammals (Fellermann; Stange, 2001). In the intestine, besides the enterocytes, the β-defensins were found in the goblet and Paneth cells (Cobo; Chadee, 2013). The role of β-defensins 1 and 2 has been investigated in the gastrointestinal tract of humans (Zilbauer et al.,

2005; Cobo; Chadee, 2013) and swine (Veldhuizen et al., 2009; Wan et al., 2013). It was reported that their antimicrobial activities are important to protect the intestinal mucosa against pathogens and regulation of commensal microbiota, besides performing functions associated to phagocytosis, neutrophil recruitment, regulation of pro-inflammatory and anti-inflammatory cytokines, facilitation of maturation and antigen uptake by dendritic cells (Yang et al., 2002; Cobo; Chadee, 2013). The balance in the production of pro-inflammatory, anti-inflammatory cytokines and defensins is essential to intestinal homeostasis, protection against infectious agents, intestinal inflammation, cancer development and harmful effects of natural food contaminants as mycotoxins (Szkaradkiewicz et al., 2009; Cobo; Chadee, 2013; Pinton, Oswald, 2014).

Deoxynivalenol (DON) and fumonisin B1 (FB1) are mycotoxins produced by *Fusarium* species and represent a health risk factor to humans and animals due to the worldwide occurrence as natural contaminants of cereals (Streit et al., 2013). Enterocytes and immune cells of the intestine are targets for the action of the DON and FB1 (Pinton; Oswald, 2014). Several studies have shown that these mycotoxins alter the expression of pro-inflammatory and anti-inflammatory cytokines (Bouhet; Oswald, 2007; Bracarense et al., 2012; Cano et al., 2013), compromising the intestinal immunological response (Pinton; Oswald, 2014). The immunotoxic effects of DON and FB1 are associated with protein synthesis inhibition through binding to the 28S ribosomal RNA peptidyltransferase site (Pestka, 2008) and inhibition of the ceramide synthase, blocking the synthesis of the sphingolipids (Soriano et al., 2005), respectively. Moreover, both mycotoxins act on mechanisms associated with the regulation of cytokines expression as mitogen-activated protein kinases (MAPKs) (Pinelli et al., 1999; Pinton et al., 2010) and cyclooxygenase-2 (Cox-2) (Silva et al., 2014) expression. However, the effects of DON and FB1 on the intestinal β-defensins expression are not well established.

As such, the search to inhibit or minimize the toxic effects of mycotoxins has been growing worldwide with the objective to improve food quality and nutritional aspects. Previous researches have demonstrated the importance of nutraceutical foods as IP6 in the prevention and treatment of several diseases (Moraes; Colla, 2006; Khatiwada et al., 2011; Mazzio et al., 2017). IP6 is a natural antioxidant widely present in cereals, legumes, nuts, oils seeds, spores, needles and pollen (Graf; Eaton, 1990). The antioxidant action of IP6 was associated with its capacity to inhibit the production of radical oxygen species (ROS) (Graf; Eaton, 1985). In the intestine, IP6 inhibits the development of colon cancer (Norazalina et al, 2010; Schroterová et al., 2010), the secretion of pro-inflammatory cytokines in inflammatory diseases (Wawszczyk et al., 2012; Kapral et al., 2014), and modulates the luminal microbiota (Okazaki et al., 2015; Sekita et al., 2016). To the best of authors' knowledge, there are no previous studies focusing on the immunological effects of IP6 in intestine exposed to mycotoxins.

Pigs have morphological, physiological and immunological similarities with the human gastrointestinal tract as well as similarities in the absorption of IP6 (Schlemmer et al., 2001), making them an excellent experimental model. Our research group seeks to elucidate the mechanisms involved in the toxic effects of DON and FB1. In addition, we are interested in investigating natural substances, such as IP6 that may inactivate or minimize the toxic effects of mycotoxins. Therefore, this study investigated the effects of IP6 on the expression of cytokines and defensins in the jejunum of pigs exposed to DON and FB1 using an *ex vivo* model.

**Material and methods**

*Animals*

Five 24-days-old crossbred (Landrace x Large White X Duroc) piglets (7.9 kg ± 0.72) were used in the present study. All animal experimentation procedures were performed in accordance with the ethics committee on the use of animals (CEUA/UEL/Brazil-process n° 4117.2014.05).

*Phytic acid*

Phytic acid (inositol hexaphosphoric acid) dodecasodium salt from rice (MW: 819) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The salt was dissolved in distilled water and the pH was adjusted to 7.2 before the solution was passed through a membrane filter. The resultant solution was stored at -20 °C before dilution in explant culture media. The IP6 concentration of 2.5 mM and 5 mM used in this study were chosen according a preview experiment with explants of jejunum swine (Silva et al., 2014).

*DON and FB1 mycotoxins*

The purified DON (MW: 296.32) and FB1 (MW: 721.83) mycotoxins were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Cayman Chemical Company (Michigan, USA), respectively. The mycotoxins were dissolved in ultrapure water at a final dilution of 10 µM for DON and 70 µM for FB1 and stored at 4 °C. The concentration of mycotoxins used was equivalent to 3 mg $kg^{-1}$ feed and 50.5 mg $kg^{-1}$ feed for DON and FB1, respectively.

*Jejunal explants technique*

The piglets were euthanized by the administration of acepromazine 1% (0.1 mL/10 Kg) IM, sodium pentobarbital (40 mg/Kg) IV and, subsequently, KCl 15% solution IV. The jejunum was rapidly excised, and samples 5 cm in length were collected, washed with buffer solution and opened longitudinally. The explants were collected using a biopsy punch (8 mm).

From each animal, three jejunal explants without the serosa layer were collected to each treatment (21 explants were sampled and placed in 6 well plates (three explants/well) with 3 mL of the following treatments: control (A) and (B and C groups) - only culture media (DMEM- Dulbecco's modified Eagle's medium (Gibco®) plus penicillin/streptomycin (1.25 µL/mL-Gibco®), gentamicin (10 µL/mL-Novafarma®), fetal bovine serum (100 µL/mL-Invitrogen®) and L-glutamine (0.4 µL/mL- Sigma Aldrich®); culture media with IP6 2.5 mM (D and E) and culture media with IP6 5 mM ( F and G). The explants were incubated at 37°C under orbital shaking. After one hour, DON (10 µM) was added in the B, D, F and FB1 (70 µM) in the C, E and G and treatments. The explants were returned to incubation by orbital shaking for an additive three hours. After the incubation period, the explants of each treatment were collected, flash-frozen in liquid $N_2$ and stored at -80°C until processed for determination of cytokine mRNA.

*Determination of the expression of mRNA encoding for cytokines by real-time PCR*

The sequences and concentration of the primers (Invitrogen®) used in the present study are detailed in Table 1. The jejunal explants tissue of each treatment was homogenized and the total RNA was extractetd using the Trizol (Invitrogen®) according to the manufacturer's instructions. Concentration, integrity and quality of RNA were determinated spectrophothometrically. The reverse transcription of the RNA was perfomed using M-MLV RT (Invitrogen®) and random primers (2 min at 37°C, 1h at 37°C and 15 min at 65°C). Real-time PCR assays were performed on 15 ng cDNA (RNA equivalent) in 20µl volume reaction per well using the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen®) and the thermocycler Rotor-Gene Q 5 Plex-HRM (Qiagen®).

The amplification conditions were as follows: 95°C for 10 min followed by forty cycles of 95°C for 15s and 60°C for 1 min. RNA non-reverse transcript was used as a non-template control to verify that no genomic DNA amplification signal existed. Specificity of

PCR products was checked at the end of the reaction by analyzing the curve of dissociation. The sequences and concentration of the primers used are detailed in Table 1. Amplification efficiency and initial fluorescence were determined using the DART-PCR method. The values obtained were then normalized by the housekeeping gene ribosomal protein L32 (RPL 32) and the gene expression was calculated relative to the control group, as previously described (Devriendt et al., 2009).

*Statistical Analysis*

Data are present as means with their standard errors and were analyzed using the software R (2.11.1.) One-way analysis of variance (ANOVA) followed by a multiple comparison procedure (Duncan's test) was used for statistical analysis. P values of $\leq 0.05$ were considered significant.

## Results

To evaluate the effect of IP6 on the expression of cytokines and β-defensins on porcine intestine exposed to DON and FB1 we quantified the gene expressions of coding for six cytokines (IL-1β, IL-6, Il-8, IL-10, IFN-γ and TNF-α) and β-defensins 1 and 2 (pBD-1 and pBD-2) as described in Table 2.

The jejunal explants exposed to DON showed a significant increase in IL-1β and IL-8 and a decrease in IL-6, IL-10, IFN-γ and pBD-2 expression ($p<0.05$). No significant reduction was also observed in the expression of TNF-α (25%) and pBD-1 (84%). On the other hand, when explants were pre-treated with 5 mM of IP6, the expression of IL-8 was significantly reduced and pBD-1 and pBD-2 showed a significant increase in their expressions when compared to DON exposure ($p<0.05$). The presence the IP6 reduced in 53% the expression of IL-1β in explants exposed to DON, but with no significance ($p>0.05$). The expression of TNF-α showed no significant change in explants exposed to DON; however, a significant reduction was observed in explants exposed to DON+IP6 5 mM when compared to the

control ($p<0.05$). The presence of IP6 2.5mM and 5 mM increased non-significantly the expression of IL-6 (two folds) and IL-10 (seven folds) compared to DON treatment.

The effects of FB1 on the intestinal immunological response were characterized by a significant increase in the expression of IL-1β and a decrease in the levels of IL-6, IL-8, IL-10, IFN-γ, TNF-α and pBD-1 ($p<0.05$). The presence of IP6 5 mM induced a significant increase in the expression of TNF-α compared with FB1 ($p<0.05$). In addition, IP6 5 mM reduced the expression of IL-1β (six folds) compared with FB1 alone and increased the expression of IL-6 (two folds), IL-8 (three folds), IL-10 (100 folds), IFN- γ (five folds) and pBD-1 (three folds).

**Discussion**

In the present study, we investigated the effect of IP6 on the response of pro- and anti-inflammatory cytokines and β-defensins expression in jejunal explants exposed to DON and FB1. The intestinal morphological and immunological changes induced by these mycotoxins have been established (Grenier et al., 2011; Bracarense et al., 2012; Silva et al., 2014). However, the effect of DON and FB1 on the expression of cytokines, chemokines and defensins in the intestine are not yet largely investigated. Efforts to inhibit and minimize the toxic effects of mycotoxins have been an area of research interested for our group (Grenier et al., 2012; Silva et al., 2014; Pierron et al., 2016). Although several mechanisms for the anticancer action of IP6 have been elucidated (Tantivejkul et al., 2003; Vucenik; Shamsuddin, 2006; Bizarri et al., 2016; Silva et al., 2016), there are few data to explain its effects on cytokine genes expression in intestine (Wawszczyk e tal., 2012; Kapral et al., 2013). In addition, there are no data about the effect of IP6 on the intestinal defensins expression. In this study, a protective effects of IP6 on immunological changes induced by DON and FB1 in the intestinal tissue was observed.

The intestinal samples submitted to DON treatment showed a significant increase on IL-1β (2.5 fold) and IL-8 (3 fold) expression, similarly to previous reports (Bracarense et al., 2012; Cano et al., 2013). The increase of both cytokines was associated to the development of intestinal cancer development and inflammatory bowel diseases (Jijon et al., 2002; Sasaki et al., 2002; Lewis et al., 2006; Rizzo et al., 2011). Previous studies about immunological changes induced by DON suggest that it may play a role in intestinal inflammatory diseases due to the overexpression of pro-inflammatory cytokines (Cano et al., 2013; Alassane-Kpembi et al., 2016). The decrease of IL-10 expression observed suggest that DON may inhibit anti-inflammatory cytokines, since the main function of IL-10 is to limit and terminate inflammatory responses (Szkaradkiewicz et al., 2009). Therefore, the modulation of IL-10 expression by DON can induce a persistent intestinal inflammatory response with consequent changes on epithelial junction protein expression and increase of intestinal susceptibility to microorganism infections as reported previously (Pinton and Oswald, 2014; Alizadeh et al., 2015; Reddy et al., 2018). In the present study, we observed a decrease of IL-6, IFN-γ and TNF-α differing to previous studies (Bracarense et al., 2012; Cano et al., 2013; Alizadeh et al., 2015). These differences may be related to concentration/dose, duration of the exposure to DON and experimental model, since these factors may influence on mycotoxins effects (Prelusky et al., 1988).

Explants exposed to the pre-treatment with IP6 presented decrease of the IL-8, IL-1β and TNF-α expression, and increase of IL-6, IFN-γ and anti-inflammatory IL-10 expression. These results showed the IP6 may modulate the immunological changes induced to DON similar to previous studies using human colonic epithelial cells (Caco-2) in which the IP6 exerts inhibitory control on the activation of the inflammatory pathways during carcinogenesis through decreased of TNF-α, IL-6 and IL-8 (Bizzarri et al., 2016; Wawszczyk et al., 2012).

In the present study, exposure to FB1 induced a decrease in pro-inflammatory cytokines expression, excepting IL-1β that increased. These results differ from others studies where an increase in pro-inflammatory cytokines was reported (Bracarense et al., 2012; Halloy et al., 2005). Probably, these differences are related to the doses and period of exposure that may influence the immunological *status*, inducing an immunosuppressive or immunostimulatory response. The FB1-induced decrease observed in most of the cytokines evaluated suggest an immunotoxic effect, contributing with a reduction in immune cells recruitment and an increased susceptibility to intestinal infections (Oswald et al., 2003). The explants exposed previously to IP6 showed an increase of IL-6, IL-8, IFN-γ, IL-10 expression and decrease the IL-1β. Similar to observed in explants exposed to DON, the IP6 regulated the immunological changes induced by FB1. Our results evidenced that IP6 modulate the cellular mechanisms and microenvironment to prevent the tissue lesion as observed in other studies (Silva et al., 2014; Zadjel et al., 2013; El-Saad and Mahmoud, 2007; Vucenik and Shamsuddin, 2006).

The β-defensins are peptides produced by epithelial cells that exert an important role in intestinal inflammatory diseases and infections, since its expression may be stimulated by microbial components and pro-inflammatory cytokines (Cobo et al., 2013). The porcine pBD-1 is a homologue of human β-defensin 2 (hBD-2) (Dybvig et al., 2011) and pBD-2 is considered an orthologue of human β-defensin 1 (hBD-1). In pigs the immunological response against infections in the respiratory system (Elahi et al., 2006) and intestine (Velduizen et al., 2009) were associated with these peptides. In humans, the pBD-2 production decreased the inflammation and mucosal lesions in colitis (Han et al., 2015). However, the effects of mycotoxins on β-defensins expression are not well established. In an *in vitro* study using IPEC-2 cells, the exposure to DON (2 μM) and FB1 (40 μM) for 48 hours induced an increase in pBD-1 and pBD-2 expression (Wan et al., 2013). In our study, we

observed that both mycotoxins induced significant decreases in expression of defensins. DON (10μM) reduced the expression of pBD-2 (99%) and FB1 (70 μM) the pBD-1(99%) expression. These controversial results are probably related to the different doses used and mainly to period of exposure to the mycotoxin. The *ex vivo* model used in this study was chosen because maintains the complex patterns of cellular differentiation seen *in vivo* (Randall et al., 2011). Therefore, we believe that our results are closer to that occurs in *in vivo* exposure to mycotoxins. Changes in the regulation of the β-defensins expression induced by DON and FB1 may cause modifications in the homeostatic state of microbiota on the mucosal epithelium, increasing the susceptibility to intestinal infections.

There are no data about the action of IP6 on the intestinal production of β-defensins and the effects on the anti-inflammatory response are not well established. In this study, the modulatory effects of IP6 on the expression of cytokines and β-defensins in jejunal explants exposed to DON and FB1 were more evident in the concentration of 5 mM, similarly to previous reports (Kapral et al., 2010; Silva et al., 2014) that demonstrated a protective effect of IP6 associated to higher doses.

In the explants exposed to DON, the pre-treatment with IP6 increased the levels of pBD-1 and 2. In the explants exposed to FB1, the IP6 induced an increase in the expression of pBD-1 and decrease pBD-2. Interestingly, the effects of IP6 were more evident in explants exposed to DON, probably t is related to the antioxidant action of IP6 on the higher oxidative stress and ROS generation induced by DON compared to FB1.

In a previous study, we showed that IP6 induced a protective effect on the jejunal mucosa of pigs exposed to DON and FB1 by the recovery of intestinal morphology, increase of E-cadherin expression and decrease of cell apoptosis and expression of Cox-2 (Silva et al., 2014). The present study demonstrated that IP6 also exerts a protective and modulatory effect on the intestinal immunology of piglets exposed to DON and FB1.

The beneficial effects of IP6 are associated with the antioxidant action inhibiting ROS production through Fenton reaction (Graf; Eaton, 1985). Besides, IP6 also modulates the activation of MAPKs and NF-κB (Zhang et al., 2015), cell proliferation and apoptosis signal transduction (Jenab; Thompson, 2000; Silva et al., 2014), expression of Cox-2 (Silva et al., 2014), cellular differentiation and morphology preservation. In addition, IP6 may induce an increase in *Lactobacillus* spp. and a decrease in intestinal pathogenic bacteria (Okazaki et al., 2014; Sekita et al., 2016), promoting advantageous effects through modulation of intestinal microbiota.

In conclusion, the present study demonstrated that DON and FB1 induce changes in the expression of intestinal cytokines and β-defensins. These immunological alterations can compromise the intestinal homeostasis and increase the susceptibility to development of intestinal diseases. Intestinal exposure to IP6 led to a beneficial modulation on immunological changes induced by the mycotoxins, contributing to the maintenance of intestinal health. Therefore, the ingestion of IP6 may help the intestinal homeostasis and microbiota, improve the absorption of nutrients and the defense against infectious agents and toxins as mycotoxins. Further studies are necessary to elucidate the interaction between mycotoxins and IP6 on the intestinal immunological response focusing on the β-defensins and other antimicrobial peptides.

## Acknowledgements

This study was supported by a grant from CNPq (474691/2012-8) and Fundação Araucária, Brazil.

## References

Alizadeh A, Braber S, Akbari P, Garssen J, Fink-Gremmels J. 2015. Deoxynivalenol affects markers of gut health after low-dose, short-term exposure of growing pigs. Toxins 7: 2071-2095.

Basso K, Gomes F, Bracarense AP. 2013. Deoxynivalenol and fumonisin, alone or in combination, induce changes on intestinal junction complexes and in E-cadherin expression. Toxins 5: 2341-2352

Becker C, Reiter M, Pfaffl MW, Meyer HHD, Bauer J, Meyer KHD. 2011. Expression of immune relevant genes in pigs under the influence of low doses of deoxynivalenol (DON). Mycotoxin Res 27: 287–293.

Bevins CL, Martin-Porter E, Ganz T. Defensins and innate host defense of the gastrointestinal tract. Gut 45: 911-915.

Bizzarri M, Dinicola S, Bevilacuq A, Cucina A. 2016. Broad spectrum anticâncer activity of myo-inositol and inositol hexakisphosphate. Int J Endocrinol 2016: 5616807.

Bouhet S, Oswald IP. 2007. The intestine as a possible target for fumonisin toxicity. Mol Nutr Food Res 51: 925-931.

Bouhet S, Oswald IP. 2005. The effects of mycotoxins, fungal food contaminants, on the intestinal epitelial cell-derived innate imune response. Vet Immunol Immunopathol 108: 199-209.

Bouhet S, Oswald IP. 2007. The intestine as a possible target for fumonisin toxicity. Mol Nutr Food Res 51: 925-931.

Buedas FJM, Sanchis CP, Perello BJ, Grases FF. 2016. Pant phosphates, phytate and pathological calcifications in chronic kidney disease. Nefrol 16: 30115-30121.

Bracarense A-PFL, Lucioli J, Grenier B, Pacheco GD, Moll W-D, Schatzmyr G. 2012. Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, inducesmorphological and immunological changes in the intestine of piglets. Br J Nutr 107: 1776-1786.

Cano PM, Seeboth J, Meurens F, Cognie J, Abrami R, Oswald IP, Guzylack-Piriou L. 2013. Deoxynivalenol as a new factor in the persistence of intestinal inflammatory diseases: an emerging hypothesis through possible modulation of Th17-mediated response. PLoS One 8: e53647.

Cobo ER, Chadee K. 2013. Antimicrobial human β-defensins in the colon and their role in infectious and non-infectious disease. Pathogens 2: 177-192.

Cholewa K, Parfiniewicz B, Bednarek I, Swiatkowska L, Jezienicka E, Kierot J, Weglarz L. 2008. The influence of phytic acid on TNF-α and its receptors genes expression in colon cancer Caco-2 cells. Acta Pol Pharm Drug Res 65: 75-79.

Devriendt B, Gallois M, Verdonck F, Wache Y, Bimczok D, Oswald IP, Goddeeris BM, Cox E. 2009. The food contaminant fumonisin B-1 reduces the maturation of porcine CD11R1(+) intestinal antigen presenting cells and antigen-specific immune responses, leading to a prolonged intestinal ETEC infection. Vet Res 40: 40.

Dybvig T, Facci M, Gerdts V, Wilson H. 2011. Bilogical roles of the host defense peptides: lessons from transgenic animals and bioengineered tissues. Cell Tissue Res 343: 213-225.

Elahi S, Buchana RM, Attah-Poku S, Townsend HGG, Babiuk LA, Gerdts V. 2006. The host defense peptide beta-defensin 1 confers protection against Bordetella pertussis in newborn piglets. Infect immune 7: 2338-2352.

Fellermann K, Stange EF. 2001. Defensins- innate immunity at the epithelial frontier. Eur J Gastroneterol Hepatol 13: 7771-776.

Frye M, Bargon J, Lembcke B, Wagner TO, Gropp R. 2000. Differential expression of human alpha- and beta-defensins mRNA in gastrointestinal epithelia. Eur J Clin Investig 30: 695–701.

Graf E, Eaton JW. 1990. Antioxidant functions of phytic acid. Free Rad Biol Med 8: 61-69.

Grenier B, Loureiro-Bracarense AP, Lucioli J, Pacheco GD, Cossalter AM, Moll WD, Schatzmayr G, Oswald IP. 2011. Individual and combined effects os of subclinical doses of deoxynivalenol and fumonisins in piglets. Mol Nutr Food Res 55: 761-771.

Gu M, Raina K, Agarwal R. 2010. Inositol hexaphosphato down-regulates constitutive and ligand-induced mitogenic and cell survival signaling and causes caspase-mediated apoptotic death of human prostate carcinoma PC-3 cells. Mol Carcinog 40: 1-12.

Han F, Zhang H, Xio X, Xiong H, Song D, Zong X, Wang Y. 2015. Porcine β-defensin 2 attenuates inflammation and mucosal lesions in dextran sodium sulfate-induced colitis. J Immunol 194: 1882-1893.

Halloy DJ, Gustin PG, Bouhet S, Oswald IP. 2005. Oral exposure to culture material extract containing fumonisins predisposes swine to the development of pneumonitis caused by *Pasteurella multocida*. Toxicol 213: 34-44.

Huang S, Xie K, Bucana CD, Ullrich SE, Bar-Eli M. 1996. Interleukin 10 suppresses tumor growth and metastasis of human melanoma cells: potential inhibition of angiogenesis. Clin Cancer Res 2: 1969-1979.

Jenab PM, Thompson LU. 2000. Phytic acid in wheat bran affects colon morphology, cell differentiation and apoptosis. Carcinogenesis 21: 1547-1552.

Jijon HB, Panenka WJ, Madsen KL, Parsons HG. 2002. MAPkinases contribute to IL-8 secretion by intestinal epithelial cells via a posttranscriptional mechanism. Am J Physiol Cell Physiol 283: 31-41.

Jung HC, Eckmann L, Yang SK, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF. 1995. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J Clin Invest 95: 55-65.

Kapral M, Sosnicki S, Wawszczyk J, Weglarz L. 2014. Influence of inositol hexaphosphato on the expression of selected proliferation markers in IL-1β-stimulated intestinal epithelial cells. Acta Pol PharmDrug Res 71: 97-993.

Kapral M, Wawszczyk J, Smolik S, Weglarz L. 2010. Transcriptional regulation of inerleukin 6 and its receptor in colon cancer cells by phytic acid. Acta Pol Pharm Drug Res 67: 701-705.

Lewis AM, Varghese S, Xu H, Alexander HR. 2006. Interleukin-1 and cancer progression: the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment. J Transl Med. 4: 48.

Liu J, Simmons SO, Pei R. 2014. Regulation of IL-8 promote activity by verruccarin A in human monocytic THP-1 cells. J Toxicol Environ Health 77: 1125-1140.

Meissonnier GM, Pinton P, Laffitte J, Cossalter AM, Gong YY, Bertin G, Galtier P, Oswald IP. 2008. Immunotoxicity of aflatoxina B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol Appl Pharmacol 231: 142-149.

Norazalina S, Norhaizan ME, Hairuszah I, Norashareena MS. 2010. Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymethane-induced colon carcinogenesis in rats. Exp Toxicol Pathol 62: 259-268.

Meurens F, Berri M, Auray G, Melo S, Levast B, Virlogeux-Payant I, Chevaleyre C, Gerdts V, Salmon H. 2009. Early immune response following *Salmonella enterica* subspecies enterica serovar Typhmurium infection in porcine jejunal gut loops. Vet Res 40: 5.

Obata T, Nakashima M. 2016. Phytic acid suppress ischemia-induced hydroxyl radical generation in rat myocardiu. Eur J Pharmacol 774: 20-24.

Okazaki Y, Katayama T. 2015. Detary phytic acid modulates characteristics of the colonic luminal environment and reduces serum levels of proinflammatory cytokines in rats fed a high-fat diet. Nut Res 34: 1085-1091.

Oswald IP. 2006. Role of intestinal epithelial cells in the innate immune defense of the pig intestine. Vet Res 37: 359-368.

Oswald IP, Desautels C, Laffitte J, Fournout S, Peres SY, Odin M, Le Bars P, Le Bars J, Fairbrother JM. 2003. The mycotoxin, fumonisin B1, increases intestinal colonization by pathogenic Escherichia coli in pigs, Appl. Environ Microbiol 69: 5870 –5874.

Pestka JJ. 2008. Mechanisms of deoxynivalenol-induced gene expression and apoptosis. Food Addit Contam A Chem Anal Control Expo Risk Asses 25: 1128-1140.

Pierron A, Mimoun S, Murate LS, Loiseau N, Lippi Y, Bracarense APFRL, Schatzmayr G, He JW, Zhou T, Moll W-D, Oswald I. Microbial biotransformation of DON: molecular basis for reduced toxicity. Sci Rep 6: 29105, 2016.

Pinelli E, Poux N, Garren L, Pipy B, Castegnaro M, Miller DJ, Pfohl-Leszkowicz A. 1999. Activation of mitogen-activated protein kinase by fumonisin B(1) stimulates cPLA(2) phosphorylation, the arachidonic acid cascade and cAMP production. Carcinogenesis, 20: 1683-1688.

Pinton P, Braicu C, Nougayrede JP, Laffitte J, Taranu I, Oswald IP. 2010. Deoxynivalenol impairs porcine intestinal barrier function and decreases the protein expression of claudin-4 through a mitogen activated protein kinase dependent mechanism. J Nutr 140: 1956-1962.

Pinton P, Nougayrède JP, Del Rio JC, Moreno C, Marin DE, Ferrier L, Bracarense AP, Kolf-Clauw M, Oswald IP. 2009. The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicol Appl Pharmacol 237: 41-48.

Pinton P, Oswald IP. 2014. Effect of deoxynivalenol and other Type B trichothecenes on the intestine: a review. Toxins 6: 1615-1643.

Prelusky DB, Hartin KE, Trenholm HL, Miller JD. Pharmacokinetic fate of 14C-labeled deoxynivalenol in swine. Fundam Appl Toxicol 10: 276-286, 1988.

Randall KJ, Turton J, Foser JR. 2011. Explant culture fo gastrointestinal tissue: a review of methods and applications. Cell Biol Toxicol 27: 267-284.

Royaee AR, Husmann RJ, Dawson HD, Calzada-Nova G, Schnitzlein WM, Zuckermann FA, Lunney JK. 2004. Deciphering the involvement of innate immune factors in the development of the host response to PRRSV vaccination. Vet Immunol Immunopathol 102: 199-216.

Sasaki Y, Tanaka M, Kudo H. 2002. Differentiation between ulcerative colitis and Crohn's disease by a quantitative immunohistochemical evaluation of T lymphocytes neutrophils, histiocytes and mast cells. Pathol Int 52: 277-285.

Schroterová L, Hakowá P, Rudolf E, Cervinka M. 2010. Effect of phytic acid and inositol on the proliferation and apoptosis of cells derived from colorectal carcinoma. Oncol Rep 23: 787-793.

Schlemmer U, Jany KD, Berk A, Schulz E, Rechkemmer G. 2001. Degradation of phytate in the gut of pigs-pathway of gastro-intestinal inositol phosphate hydrolysis and enzymes involved. Arch Tierernahr 55: 255-280.

Sekita A, Okazaki Y, Katayama T. 2016. Dietary phytic acid prevents fatty liver by reducing expression of hepatic lipogenic enzymes and modulates gut microflora in rats fed a high sucrose-diet. Nutr 32: 720-722.

Selsted ME, Millers IS, Henschen AH, Quellette AJ. 1992. Enteric defensins: antibiotic peptide components of intestine hos defense. J Cell Biol 118: 929-936.

Silva EO, Gerez JR, Drape TC, Bracarense APFRL. 2014. Phytic acid decreases deoxynivalenol and fumonisin B1-induced cahnges on swine jejunal explants. Toxicol Rep 1: 284-292.

Silva EO, Bracarense APFRL. Phytic acid: from antinutritional to multiple protection factor of organic systems. J Food Sci 81: 1357-1362.

Soriano JM, González L, Catalá AI. 2005. Mechanism of action of sphingolipids and their metabolities in the toxicity of fumonin B1. Prog Lipid Res 44: 345-356.

Stadnyk AW. 2002. Inestinal epithelial cell as a source of inflammatory cytokines and chemokines. Can J Gatroenterol 16: 241-246.

Streit E, Naehrer K, Rodrigues I, Schatzmayr G. 2013. Mycotoxin occurrence in feed and feed raw materials worldwide: long-term analysis with special focus on Europe and Asia. J Sci Food Agric 93: 2892-2899.

Szkaradkiewicz A, Marciniak R, Chudzicka-Strugala I, Wasileska A, Drews M, Majewski P, Karpiński T, Zwoździak B. 2009. Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. Arch Immunol Ther Exp 57: 291-294.

Tantivejkul K, Vucenik I, Shamsuddin AM. 2003. Inositol hexphosphate (IP6) inhibits key events of cancer metastasis: I. Invtro studies of adhesion, migration and invasion of MDA-MB 231 human breast cancer cells. Anticancer Res 23: 671-679.

Vucenik I, Shamsuddin AM. 2006. Protection against cancer by dietary $IP_6$ and inositol. Nutr Cancer 55: 109-125.

Veldhuizen EJA, Koomen I, Ultee T, Van Dijk A, Haagsman HP. 2009. *Salmonella serovar* specific upregulation of porcine defensins 1 and 2 in a jejunal epithelial cell line. Vet Microbiol 136: 69-75.

Wan M L-Y, Woo C-S J, Allen KJ, Turner PC, El-Nezami H. 2013. Modulation of porcine β-defensins 1 and 2 upon individual and combined Fusarium toxin exposure in a swine jejunal epithelial cell line. Appl Environ Microbiol 79: 2225-2232.

Watson AJ, Hughes KR. 2012. TNF-α-induced intestinal epithelial cell shedding: implications for intestinal barrier function. Ann New York Acad Sci 1258: 1-8.

Wawszczyky J, Orchel A, Kapral M, Holler A, Weglarz L. 2012. The effect of phytic acid on the expression of NF-κB, IL-6 and IL-8 in IL-1β-stimulated human colonic epithelial cells. Acta Pol Pharm Drug Res 69: 1313-1319.

Wawszczyky J, Orchel A, Kapral M, Holler A, Weglarz L. 2012. Phytic acid down-regulates IL-8 secretion from colonic epithelial cells by influencing mitogen-activated protein –kinase signaling pathway. Acta Pol Pharm Drug Res 69: 1276-1282.

Wawszczyky KJ, Kapral M, Lodowska J, Jesse K, Hollek A, Weglarz L. 2015. Antiproliferative effect of inositol hexaphosphato on human skin melanoma cells *in vitro*. Acta Pol Pharm Drug Res 72: 895-900.

Weglarz L, Wawszczyk J, Orchel A, Jaworska-Ki KM, Dzierzeicz Z. 2006. Phytic acid modulates in vitro IL-8 and IL-6 release from colonic epithelial cells stimulate with LPS and IL-1β. Dig Dis Sci 52:93-102.

Yang D, Biragyn A, Kwak LW, Oppenheim JJ. 2002. Mammalian defensins in immunity: more than just micorbiocidal. Trends Immunol 23: 39-48.

Zhang LY, Hou L, Zhang L, Zhang J, Wang Y, Liu C, Xu P, Liu L, Gai X, Lu T. 2015. Phytic acid attenuates inflammatory responses and the levels of NF-κB and p-ERK in MPTP-induced Parkinson's disease model of mice. Neurosci Lett 597: 132-136.

Zilbauer M, Dorrell n, Boughan PK, Harris A, Wren BW, Bajaj-Elliott M. 2005. Intestinal innate immunity to *Campylobacter jejuni* results in induction of bactericidal human beta-defensins 2 and 3. Infect Immun 73: 7281-7289.

Zins K, Abraham D, Sioud M, Aharinenad S. 2007. Colon cancer cell-derived tumor necrosis factor-alpha mediates the tumor growth-promoting response in macropahges by up-regulating the colony-stimulating factor-1 pathway. Cancer Res 67: 1038-1045.

**Table 1.** Nucleotide sequence of primers for real-time PCR

| Gene | Primers sequences | GenBank no. | Reference |
|---|---|---|---|
| RPL-32 | F (300 nM) TGCTCTCAGACCCCTTGTGAAG<br>R (300 nM) TTTCCGCCAGTTCCGCTTA | NM_001001636 | Pinton et al., 2010 |
| IL-1β | F (300 nM) GAGCTGAAGGCTCTCCACCTC<br>R (300 nM) ATCGCTGTCATCTCCTTGCAC | NM_001005149 | Devriendt et al., 2009 |
| IL-6 | F (300 nM) GGCAAAAGGGAAAGAATCCAG<br>R (300 nM) CGTTCTGTGACTGCAGCTTATCC | NM_214399 | Grenier et al., 2011 |
| IL-8 | F (300 nM) GCTCTCTGTGAGGCTGCAGTTC<br>R (900nM) AAGGTGTGGAATGCGTATTTATGC | NM_213867 | Grenier et al.,2011 |
| IL-10 | F (300 nM) GGCCCAGTGAAGAGTTTCTTTC<br>R (300 nM) CAACAAGTCGCCCATCTGGT | NM_214041 | Bracarense et al., 2012 |
| IFN-γ | F (300 nM) TGGTAGCTCTGGGAAACTGAATG<br>R (300 nM) GGCTTTGCGCTGGATCTG | NM_213948 | Royaee et al., 2004 |
| TNF-α | F (300 nM) ACTGCACTTCGAGGTTATCGG<br>R (300 nM) GGCGACGGGCTTATCTGA | NM_214022 | Meissonnier et al., 2008 |
| pBD-1 | F (141 nM) CTCCTCCTTGTATTCCTCCT<br>R (141 nM) GGTGCCGATCTGTTTCAT | NM_213838 | Wang et al., 2013 |
| pBD-2 | F (148 nM) GACTGTCTGCCTCCTCTC<br>R (148 nM) GGTCCCTTCAATCTGTTG | NM_214442 | Wang et al., 2013 |

RPL-32, ribosomal protein L32; F- forward; R- reverse

**Table 2.** Effect of IP6 on the mRNA expression of cytokines in swine jejunum exposed to DON and FB1.

| **Cytokine** | **Control** | | **DON** | | **DON+IP6 2.5 mM** | | **DON+IP6 5 mM** | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| IL-1β | 1.00[a] | 0.99 | 2.66[b] | 2.59 | 3.25[ab] | 3.22 | 1.27[ab] | 1.25 |
| IL-6 | 1.00[a] | 0.99 | 0.21[b] | 0.21 | 0.43[ab] | 0.26 | 0.44[ab] | 0.26 |
| IL-8 | 1.00[a] | 0.60 | 3.21[b] | 3.57 | 0.55[ab] | 0.34 | 0.62[a] | 0.62 |
| IL-10 | 1.00[a] | 1.00 | 0.07[b] | 0.07 | 0.61[ab] | 0.34 | 0.55[ab] | 0.27 |
| IFN-γ | 1.00[a] | 0.38 | 0.67[b] | 0.33 | 0.75[ab] | 0.38 | 0.76[ab] | 0.38 |
| TNF-α | 1.00[a] | 1.00 | 0.75[ab] | 0.75 | 0.70[ab] | 0.70 | 0.53[b] | 0.53 |
| pBD-1 | 1.00[ab] | 0.39 | 0.16[a] | 0.08 | 0.69[ab] | 0.69 | 1.72[b] | 0.61 |
| pBD-2 | 1.00[ac] | 0.99 | 0.01[b] | 0.01 | 0.56[ac] | 0.53 | 0.68[ac] | 0.45 |
| | **Control** | | **FB1** | | **FB1+IP6 2.5 mM** | | **FB1+IP6 5 mM** | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| IL-1β | 1.00[a] | 0.99 | 1.37[b] | 0.85 | 0.49[ab] | 0.44 | 0.23[ab] | 1.18 |
| IL-6 | 1.00[a] | 0.99 | 0.03[b] | 0.03 | 0.08[ab] | 0.04 | 0.06[ab] | 0.06 |
| IL-8 | 1.00[a] | 0.57 | 0.14[b] | 0.14 | 0.35[ab] | 0.30 | 0.53[ab] | 0.44 |
| IL-10 | 1.00[a] | 0.61 | 0.00[b] | 0.00 | 0.01[ab] | 0.01 | 0.01[ab] | 0.01 |
| IFN-γ | 1.00[a] | 0.06 | 0.02[b] | 0.00 | 0.08[ab] | 0.07 | 0.10[ab] | 0.09 |
| TNF-α | 1.00[a] | 0.99 | 0.00[b] | 0.00 | 0.57[ab] | 0.27 | 1.10[a] | 1.10 |
| pBD-1 | 1.00[a] | 0.98 | 0.01[b] | 0.01 | 0.03[ab] | 0.01 | 0.03[ab] | 0.03 |
| pBD-2 | 1.00[a] | 0.59 | 2.22[ab] | 1.66 | 1.18[ab] | 0.94 | 0.76[ab] | 0.40 |

Mean values with their standard errors (n 5 animals). Duncan's test.
[a,b] Mean values with unlike superscript letters (line) were significantly different for each cytokine ($p \leq 0.05$).

# 6 CONCLUSÕES

- As micotoxinas FB1 (70 μM) e DON (10 μM) induziram alterações morfológicas significativas nos explantes jejunais. Além disso, principalmente os tratamentos com DON, induziram a uma diminuição da expressão de E-caderina e aumento da apoptose, proliferação celular, expressão da cox-2 e estresse oxidativo.

- A presença de IP6 (5mM) diminuiu significativamente as alterações morfológicas, proliferação celular, apoptose, expressão de cox-2 e o estresse oxidativo nos explantes jejunais expostos a FB1 e DON. Ainda, o IP6 aumentou significativamente a expressão de E-caderina.

- As micotoxinas FB1 (70 μM) e DON (10 μM) induziram a alterações na expressão do RNAm de citocinas pró- e anti-inflamatórias e β-defensinas 1 e 2.

- O IP6, principalmente na concentração de 5 mM, modulou as alterações induzidas por FB1 e DON na expressão de citocinas pró- e anti-inflamatórias e β-defensinas 1 e 2.

# ANEXO

# ANEXO A

Universidade
Estadual de Londrina

## COMISSÃO DE ÉTICA NO USO DE ANIMAIS

**OF. CIRC. CEUA N º 034/2014** **Londrina, 09 de Março de 2014.**

**Prezada Pesquisadora,**

A CEUA/UEL reunida em 18 de Março de 2014 avaliou o projeto de pesquisa intitulado **"Efeito do ácido fítico sobre a morfologia intestinal, produção de citocinas e expressão de proteínas de junção e MAPKs em explantes intestinais de suínos expostos à Desoxinivalenol e fumosina B1"**, processo CEUA **nº 4173.2014.05**, pesquisa do Centro de Ciências Agrárias de sua responsabilidade. Esclarecidos os aspectos metodológicos solicitados o projeto está **aprovado** para execução entendendo-se que os princípios éticos postulados pelo Conselho Nacional de Controle de Experimentação Animal estão respeitados.

Serão utilizados 6 suínos da linhagem DxLWxL, idade 24 dias, peso aproximado 7,8kg sendo 3 machos e 3 fêmeas, os mesmos animais serão utilizados em outro experimento que utilizará probióticos e prebióticos ao invés do ácido fítico. Cada suíno representa uma repetição experimental. Os animais após o desmame serão aclimatizados por três dias, alimentados com ração adequada e água *ad libitum,* sendo mantidos em baias individuais na Fazenda Escola. O projeto tem como objetivo analisar os efeitos do ácido fítico sobre explantes intestinais de suínos expostos as micotoxinas desoxinivalenol e fumonisina B1 isoladas ou associadas. Após o período de desmame, eles serão eutanasiados (acepromazina 1% (0,1 mL/10kg) IM, seguindo de pentobarbital sódico (40mg/Kg) IV e solução de KCL15% IV) e coletados os fragmentos intestinais para a realização do experimento. De cada suíno serão coletados 60 fragmentos de duodeno e distribuídos como replicatas (6) nos diferentes tratamentos. Após a coleta dos fragmentos, as carcaças dos animais serão coletadas por empresa conveniada com a UEL. O processo está aprovado para execução em 37 meses.

Cumpre orientar que caso pretendam-se quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação da CEUA/UEL anteriormente à execução das modificações.

Sem mais para o momento, subscrevo-me. Cordialmente,

Waldiceu Ap. Verri Junior
Prof. Dr. Waldiceu Aparecido Verri Junior
Coordenador da CEUA/UEL

**Ilma. Sra.**
**Profa. Dra. Ana Paula Frederico R.L Bracarense**
Coordenadora do Projeto
Centro de Ciências Agrárias
Departamento de Medicina Veterinária Preventiva
Com cópia para Sra Égle Maria de Sousa (Chefe da DCA/PROPPG), Diretor(a) do Centro de Ciências Agrárias.

Campus Universitário: Rodovia Celso Garcia Cid (PR 445), km 380 - Fone (043) 3371-4000 PABX - Fax 3328-4440 - Caixa Postal 6.001 - CEP 86051-990 – Internet http://www.uel.br
Hospital Universitário/Centro de Ciências da Saúde: Av. Robert Koch, 60 - Vila Operária – Fone (043) 381-2000 PABX – Fax 337-4041 e 337-7495 - Caixa Postal 791 - CEP 86038-440
LONDRINA - PARANÁ - BRASIL