



UNIVERSIDADE ESTADUAL DE LONDRINA PARANÁ



A Reitora da Universidade Estadual de Londrina,
no uso de suas atribuições e tendo em vista a conclusão do curso de doutorado do
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL
em 04 de novembro de 2011, confere o título de

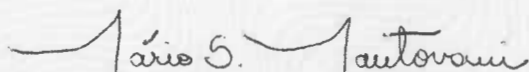
DOUTOR EM CIÊNCIA ANIMAL

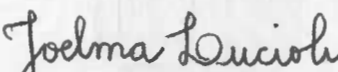
a

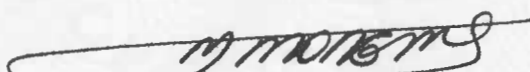
Joelma Luciola,

brasileira, natural do Estado de Santa Catarina, nascida a 15 de março de 1974, RG 29835720/SC,
e outorga-lhe o presente diploma, a fim de que possa gozar de todos os direitos e prerrogativas legais.

Londrina, 08 de março de 2012.


Prof. Dr. Mário Sérgio Mantovani
Pró-Reitor de Pesquisa e Pós-Graduação


Diplomada


Prof.ª Dra. Nádia Aparecida Moreno
Reitora

Nº 003136

RECONHECIMENTO: PORTARIA MEC
Nº 524/2008 - D.O.U Nº 82 DE
30/04/2008.

UNIVERSIDADE ESTADUAL DE LONDRINA
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

Diploma registrado sob nº 3878 Livro 03 Folha 82 de acordo com o Art. 48, da Lei nº 9.394 de 20/12/96 e Ato Executivo nº 084/09, Artigo 14 Inciso XVI de 06/11/2009 do Reitor da Universidade Estadual de Londrina.

Londrina, 08 de março de 2012.


Prof. Dr. Mário Sérgio Mantovani
Pró-Reitor de Pesquisa e Pós-Graduação

Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets

Ana-Paula F. L. Bracarense¹, Joelma Lucioli^{1†}, Bertrand Grenier^{2,3†}, Graziela Drociunas Pacheco^{1,2}, Wulf-Dieter Moll³, Gerd Schatzmayr³ and Isabelle P. Oswald^{2*}

¹Universidade Estadual de Londrina, Lab Patologia Animal, Londrina, Brazil

²INRA, UMR 1331 ToxAlim, Research Center in Food Toxicology, 180 Chemin de Tournefeuille BP 93173, 31027 Toulouse Cedex 3, Toulouse, France

³BIOMIN Research Center, Technopark 1, Tulln, Austria

(Submitted 19 April 2011 – Final revision received 2 August 2011 – Accepted 3 August 2011 – First published online 22 September 2011)

Abstract

Deoxynivalenol (DON) and fumonisins (FB) are mycotoxins produced by *Fusarium* species, which naturally co-occur in animal diets. The gastrointestinal tract represents the first barrier met by exogenous food/feed compounds. The purpose of the present study was to investigate the effects of DON and FB, alone and in combination, on some intestinal parameters, including morphology, histology, expression of cytokines and junction proteins. A total of twenty-four 5-week-old piglets were randomly assigned to four different groups, receiving separate diets for 5 weeks: a control diet; a diet contaminated with either DON (3 mg/kg) or FB (6 mg/kg); or both toxins. Chronic ingestion of these contaminated diets induced morphological and histological changes, as shown by the atrophy and fusion of villi, the decreased villi height and cell proliferation in the jejunum, and by the reduced number of goblet cells and lymphocytes. At the end of the experiment, the expression levels of several cytokines were measured by RT-PCR and some of them (TNF- α , IL-1 β , IFN- γ , IL-6 and IL-10) were significantly up-regulated in the ileum or the jejunum. In addition, the ingestion of contaminated diets reduced the expression of the adherent junction protein E-cadherin and the tight junction protein occludin in the intestine. When animals were fed with a co-contaminated diet (DON + FB), several types of interactions were observed depending on the parameters and segments assessed: synergistic (immune cells); additive (cytokines and junction protein expression); less than additive (histological lesions and cytokine expression); antagonistic (immune cells and cytokine expression). Taken together, the present data provide strong evidence that chronic ingestion of low doses of mycotoxins alters the intestine, and thus may predispose animals to infections by enteric pathogens.

Key words: Multi-contamination: Mycotoxins: Deoxynivalenol: Fumonisin: Intestine: Cellular junctions: Immunity

Mycotoxins are secondary metabolites of various fungi commonly found in feed and foodstuffs. Based on their known and suspected effects on human and animal health, aflatoxin, fumonisin, deoxynivalenol, ochratoxin A and zearalenone are recognised as the five most important agricultural mycotoxins⁽¹⁾. The toxic effects of *Fusarium* mycotoxins in animals include reduced growth, feed refusal, immunosuppression, gastrointestinal lesions, and neurological and reproductive disorders⁽²⁾.

Recent surveys^(3,4) have demonstrated the regular occurrence of low levels of multiple mycotoxins in cereals. The toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities^(5,6).

Interactions between concomitantly occurring mycotoxins can be antagonistic, additive or synergistic. Data on the combined toxic effects of mycotoxins are limited and, therefore, the actual combined health risk from exposure to mycotoxins is unknown⁽⁶⁾. Worldwide surveys on the occurrence and contamination levels of mycotoxins in raw materials also indicate that deoxynivalenol (DON) and fumonisins (FB) are the most frequently detected mycotoxins^(3,4). It was thus of interest to determine their toxic effect when present simultaneously.

The intestinal tract is the first barrier against ingested antigens, including mycotoxins and pathogenic bacteria. Following ingestion of mycotoxin-contaminated food,

Abbreviations: DON, deoxynivalenol; FB, fumonisins; MIP-1 β , macrophage inflammatory protein-1 β .

*Corresponding author: Dr I. P. Oswald, email isabelle.oswald@toulouse.inra.fr

† Both authors contributed equally to this study.

RESEARCH ARTICLE

Individual and combined effects of subclinical doses of deoxynivalenol and fumonisins in piglets

Bertrand Grenier^{1,2}, Ana-Paula Loureiro-Bracarense³, Joelma Luciolli³, Graziela Drociunas Pacheco^{1,3}, Anne-Marie Cossalter¹, Wulf-Dieter Moll², Gerd Schatzmayr² and Isabelle P. Oswald¹

¹INRA, Unité de Pharmacologie-Toxicologie, Toulouse, France

²BIOMIN Research Center, Tulln, Austria

³Universidade Estadual de Londrina, Lab Patologia Animal, Londrina, Brazil

Scope: Deoxynivalenol (DON) and fumonisins (FB) are the most frequently encountered mycotoxins produced by *Fusarium* species and most commonly co-occur in animal diets. These mycotoxins were studied for their toxicity in piglets on several parameters including plasma biochemistry, organ histopathology and immune response.

Methods and results: Twenty-four 5-wk-old animals were randomly assigned to four different groups, receiving separate diets for 5 wk, a control diet, a diet contaminated with either DON (3 mg/kg) or FB (6 mg/kg) or both toxins. At days 4 and 16 of the trial, the animals were subcutaneously immunized with ovalbumin to assess their specific immune response. The different diets did not affect animal performance and had minimal effect on hematological and biochemical blood parameters. By contrast, DON and FB induced histopathological lesions in the liver, the lungs and the kidneys of exposed animals. The liver was significantly more affected when the two mycotoxins were present simultaneously. The contaminated diets also altered the specific immune response upon vaccination as measured by reduced anti-ovalbumin IgG level in the plasma and reduced lymphocyte proliferation upon antigenic stimulation. Because cytokines play a key role in immunity, the expression levels of IL-8, IL-1 β , IL-6 and macrophage inflammatory protein-1 β were measured by RT-PCR at the end of the experiment. The expression of these four cytokines was significantly decreased in the spleen of piglets exposed to multi-contaminated diet.

Conclusion: Taken together, our data indicate that ingestion of multi-contaminated diet induces greater histopathological lesions and higher immune suppression than ingestion of mono-contaminated diets.

Received: August 25, 2010

Revised: November 6, 2010

Accepted: November 25, 2010

Keywords:

Co-contamination / Deoxynivalenol / Fumonisins / Immunity / Subclinical doses

1 Introduction

Mycotoxins are secondary metabolites of fungi that may contaminate animal feeds and human foods. They are frequently detected in grains, but also in fruits, vegetables, nuts and silages. The Food and Agricultural Organization

estimated that as much as 25% of the world's agricultural commodities are contaminated with mycotoxins and the economic losses due to mycotoxin contamination are estimated in billions of dollars annually worldwide [1]. Clinical signs caused by mycotoxins range from mortality to slow growth and reduced reproductive efficiency. Consumption

Correspondence: Dr. Isabelle P. Oswald, INRA-Laboratoire de Pharmacologie-Toxicologie, 180 chemin de Tournefeuille BP 93173, 31027 Toulouse Cedex 3, France

E-mail: isabelle.oswald@toulouse.inra.fr

Fax: +33-5-61-28-53-10

Abbreviations: APC, antigen-presenting cells; BALT, bronchiole-associated lymphoid tissue; DON, deoxynivalenol; FB, fumonisins; HE, hematoxylin-eosin; MAPK, mitogen-activated protein kinase; MIP-1 β , macrophage inflammatory protein-1 β ; OVA, ovalbumin

Toxicity of Deoxynivalenol and Its Acetylated Derivatives on the Intestine: Differential Effects on Morphology, Barrier Function, Tight Junction Proteins, and Mitogen-Activated Protein Kinases

Philippe Pinton,^{*,†} Dima Tsybulsky,^{*,†} Joelma Luciola,^{*,†,‡} Joëlle Laffitte,^{*,†} Patrick Callu,[§] Faouzi Lyazhri,[¶] François Grosjean,[§] Ana Paula Bracarense,[‡] Martine Kolf-Clauw,^{*,¶} and Isabelle P. Oswald^{*,†,1}

^{*}INRA, UMR1331, Toxalim, Research Centre in Food Toxicology, F-31027 Toulouse, France; [†]Université de Toulouse, INP, UMR1331, Toxalim, F-31000 Toulouse, France; [‡]Universidade Estadual, Departamento de Medicina Veterinária Preventiva, Londrina, Brazil; [§]Arvalis-Institut du végétal, 41100 Villerable, France; and [¶]Université de Toulouse, ENVT, INP, F-31076 Toulouse, France

¹To whom correspondence should be addressed at INRA, UMR-1331, Toxalim, 180 chemin de Tournefeuille, 31027 Toulouse cedex 3, France.
E-mail: isabelle.oswald@toulouse.inra.fr

Received May 7, 2012; accepted July 18, 2012

The intestinal epithelium is the first barrier against food contaminants and is highly sensitive to mycotoxins, especially deoxynivalenol (DON). Consumption of DON-contaminated food is associated with outbreaks of gastroenteritis. In cereals and their byproducts, DON is present together with two acetylated derivatives, 3-ADON and 15-ADON. The aim of this study was to compare the intestinal toxicity of DON and A-DONs, using non-cytotoxic doses. The toxicity was assessed using *in vitro* (intestinal epithelial cell line), *ex vivo* (intestinal explants), and *in vivo* (animals exposed to mycotoxin-contaminated diets) models. The effects were studied on cell proliferation, barrier function, and intestinal structure. The mechanism of toxicity was investigated by measuring the expression of the tight junction proteins and of phosphorylated ERK1/2, p38, and JNK, which are effectors of signaling pathway involved in cellular programs including embryogenesis, proliferation, differentiation, and apoptosis. On proliferating cells, 3-ADON was less toxic than DON, which was less toxic than 15-ADON. On differentiated cells, 15-ADON impaired the barrier function, whereas DON and 3-ADON did not have a significant effect. Similarly, *ex vivo* and *in vivo*, 15-ADON caused more histological lesions than DON or 3-ADON. At the molecular level, the 15-ADON activated the mitogen-activated protein kinases (MAPK) ERK1/2, p38, and JNK in the intestinal cell line, explants, and the jejunum from exposed animals at lower dose than DON and 3-ADON. Our results show that the higher toxicity of 15-DON is due to its ability to activate the MAPK. Given that cereal-based foods are contaminated with DON and acetylated-DON, the higher toxicity of 15-ADON should be taken into account.

Key Words: trichothecenes; acetyl-deoxynivalenol; intestine; intestinal epithelial cells; MAP Kinase; tight junction proteins.

The trichothecene mycotoxin deoxynivalenol (DON) is produced following infestation by the fungus *Fusarium*. It commonly

contaminates cereals and cereal products in human food and animal feed throughout the world, especially in temperate regions. It is not only the most commonly detected trichothecene but it may also be found at high concentrations (Canady *et al.*, 2001). DON may be produced together with two acetylated derivatives, 3-ADON and 15-ADON; and this cocontamination can reach 10–20% of the DON level and is more prevalent in maize than other cereals (European Commission, 2003).

Because of serious concerns for animal and human health, regulatory or guideline levels for DON in food and feed are in force in more than 40 countries (FAO, 2004). The toxic effects induced by DON are well characterized in all animal species, with pigs being the most susceptible (Pestka, 2010). DON can affect the immune system (Pinton *et al.*, 2008), animals' food consumption, growth, reproduction, neuroendocrine signaling, and also intestinal function (Pestka, 2010; Pinton *et al.*, 2010). In humans, there is historical evidence suggesting that DON causes acute illness and is frequently associated with outbreaks of gastroenteritis (Pestka, 2010). The intestine is the first barrier to food contaminants. Following ingestion of mycotoxin-contaminated food or feed, intestinal epithelial cells can be exposed to high concentrations of toxins (Maresca *et al.*, 2008). Highly dividing cells, such as intestinal epithelial cells, are especially sensitive to trichothecenes, and the exposure of intestinal epithelial cells to these toxins may alter their capacity to proliferate and to insure a proper barrier function. Exposure to DON can lead to impaired absorption of nutrients (Awad *et al.*, 2007) and decrease of cell proliferation or differentiation (Bensassi *et al.*, 2009; Kasuga *et al.*, 1998). The entry of luminal antigens and bacteria, normally restricted to the gut lumen, was demonstrated to be a consequence of the disruption of the barrier function in cell culture models (Maresca *et al.*, 2008) or in mice species (Li *et al.*, 2005). Recently, it was shown in pigs

Biotransformation Approaches To Alleviate the Effects Induced by *Fusarium* Mycotoxins in Swine

Bertrand Grenier,^{†,‡,§} Ana-Paula F. L. Bracarense,^{||} Heidi E. Schwartz,[⊥] Joelma Luciola,^{||} Anne-Marie Cossalter,^{†,‡} Wulf-Dieter Moll,[§] Gerd Schatzmayr,[§] and Isabelle P. Oswald^{*,†,‡}

[†]INRA, UMR 1331 ToxAlim, Research Centre in Food Toxicology, 180 Chemin de Tournefeuille BP 93173, 31027 Toulouse Cedex 3, Toulouse, France

[‡]Université de Toulouse, INP, UMR 1331 Toulouse, France

[§]BIOMIN Research Center, Technopark 1, Tulln, Austria

^{||}Universidade Estadual de Londrina, Lab. Patologia Animal, Londrina, Brazil

[⊥]Christian Doppler Laboratory for Mycotoxin Metabolism, University of Natural Resources and Life Sciences, Vienna, Department IFA Tulln, Tulln, Austria

ABSTRACT: Mycotoxin mitigation is of major interest as ingestion of mycotoxins results in poor animal health, decreased productivity, as well as substantial economic losses. A feed additive (FA) consisting of a combination of bacteria (*Eubacterium* BBSH797) and enzyme (fumonisin esterase FumD) was tested in pigs for its ability to neutralize the effects of mono- and co-contaminated diets with deoxynivalenol (DON) and fumonisins (FB) on hematology, biochemistry, tissue morphology, and immune response. Forty-eight animals, allocated into eight groups, received one of eight diets for 35 days: a control diet, a diet contaminated with either DON (3 mg/kg) or FB (6 mg/kg), or both toxins, and the same four diets with FA. Inclusion of FA restored the circulating number of neutrophils of piglets fed the FB and DON + FB diets. Similarly, FA counteracted the minor changes observed on plasma concentrations of albumin and creatinine. In lung, the lesions induced by the ingestion of FB in mono- and co-contaminated diets were no longer observed after addition of FA in these diets. Lesions recorded in the liver of pigs fed either of the contaminated diets with FA were partly reduced, and the increased hepatocyte proliferation was totally neutralized when FA was present in the co-contaminated diet. After 35 days of exposure, the development of the vaccinal response was significantly improved in animals fed diets supplemented with FA, as shown by results of lymphocyte proliferation, cytokine expression in spleen, and the production of specific Ig. Similarly, in jejunum of animals fed diets with FA, occurrence of lesions and upregulation of pro-inflammatory cytokines were much less obvious. The ameliorative effects provided by FA suggest that this approach would be suitable in the control of DON and FB that commonly co-occur in feed.

KEYWORDS: deoxynivalenol, fumonisin, co-contamination, biotransformation, vaccinal and intestinal responses

INTRODUCTION

Mycotoxins are structurally diverse low-molecular-weight metabolites produced by fungi. As secondary metabolites, they are not essential to fungi growth but may contaminate animal and human feeds at all stages of the food chain. Their global occurrence is considered to be a major risk factor affecting both human and animal health, and additionally causing substantial economic losses.¹

Among mycotoxins, deoxynivalenol (DON) and fumonisins (FB) are both produced by *Fusarium* species (mostly *F. graminearum* and *F. verticillioides*, respectively). The major problem associated with animal feed contaminated with mycotoxins is not acute disease episodes, but rather the ingestion of low level of toxins which may cause an array of metabolic, physiologic, and immunologic disturbances.^{1,2} For instance, ingestion of realistic doses of DON has been reported to alter functions of the gut epithelium, such as permeability, mucosal immunity, and nutrient utilization.^{3–6} At low doses, FB interfere with mechanisms of resistance and immune function, predisposing animals to pulmonary infections and increasing intestinal pathogen colonization.^{7–9} Besides, both DON and FB have been

reported in separate studies to impair the development of vaccinal response.^{10,11}

As evidenced by recent surveys, animals are usually exposed to more than one mycotoxin.^{12–14} The effects resulting from mycotoxin co-contamination are poorly documented, but toxicity of combinations of mycotoxins cannot be predicted from the individual toxicities of the toxins.¹⁵ With regard to the effects of DON and FB in association, a few studies demonstrated that these two toxins act in an additive manner on the induction of hepatic lesions, on the modulation of intestinal functions, or on the reduced efficacy of vaccination.^{16–19}

Good feed management practices on the farm are major goals in controlling mycotoxicosis in livestock and poultry.^{20,21} However, despite substantial efforts, prevention of mycotoxin formation in stored feeds is not always feasible. Methods based on the inclusion of feed additive to inhibit the intestinal absorption of mycotoxins have gained popularity over the past

Received: January 28, 2013

Revised: June 11, 2013

Accepted: June 11, 2013

Published: June 11, 2013

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

Martine Kolf-Clauw · Marcia Sassahara · Joelma Luciola ·
Juliana Rubira-Gerez · Imourana Alassane-Kpembi ·
Faouzi Lyazhri · Christiane Borin · Isabelle P. Oswald

Received: 17 February 2013 / Accepted: 23 April 2013 / Published online: 7 May 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Enniatins, the most prevalent emerging mycotoxins, represent an emerging food safety issue, because of their common co-occurrence with other fusariotoxins such as trichothecenes co-produced by *Fusarium spp* on field grains and because of their extensive prevalence in grains. In this study, the intestinal toxicity of enniatin B1 (ENN) alone and mixed with the most toxic trichothecene T-2 toxin (T2) was characterized by using two biological models from pig, the most sensitive species: the intestinal cell line IPEC1 (in vitro exposure) and jejunal explants (ex vivo exposure). Dose-dependent decreases in cell proliferation in IPEC1 and in the histopathological scores of explants were observed for ENN at μM -levels and for T2 at nM-levels, with IC₅₀ values for ENN of 15.8 and 29.7 μM , and for T2 of 9.3 and 15.1 nM in vitro and ex vivo, respectively. Interaction analysis by probabilistic and by determinist

approaches showed a less than additive effect both in vitro and ex vivo, at IC₅₀ values, with increasing antagonism with decreasing concentrations of toxins. The results obtained by the determinist median-effect dose analysis and by the nonlinear regression analysis were concordant. All the median-effect doses estimated for IPEC cells were included in the IC₅₀ confidence intervals of the nonlinear regression fitting. Given the occurrence of enniatins, potential synergy following the co-occurrence of enniatins and the major fusariotoxins, especially trichothecene B deoxynivalenol should be investigated.

Keywords Emerging mycotoxins · Enniatins · Gastrointestinal toxicity · Jejunal explants · Interactions · Combination index

Introduction

The increasing world population requires more and safe food in the future, but the worldwide contamination of cereals and cereal products by mycotoxins, secondary metabolites produced by filamentous fungi, is of potential concern for human and animal health. In Europe, the contamination of cereals by *Fusarium* mycotoxins raises a worrisome problem linked to climatic changes and to co-contaminations (Van Der Fels-Klerx et al. 2012). Fusariotoxins, produced by various *Fusarium spp*, are most frequently present as mixtures (Rodrigues and Nahrer 2012). They include the well-known trichothecenes, fumonisins and zearalenone chemical groups, but also the enniatins, beauvericin, moniliformin and fusaproliferin, which are frequently referred as emerging mycotoxins (Jestoi 2008). These emerging mycotoxins are usually co-produced with the well-known fusariotoxins, but have very rarely been

M. Kolf-Clauw (✉) · J. Rubira-Gerez
ENVIT, INP, UMR1331, Toxalim Research Centre in Food
Toxicology, Université de Toulouse, 31076 Toulouse, France
e-mail: m.kolf-clauw@envt.fr

M. Kolf-Clauw · M. Sassahara · J. Luciola · J. Rubira-Gerez ·
I. Alassane-Kpembi · C. Borin · I. P. Oswald
INRA, UMR1331, Toxalim, Research Centre in Food Toxicology,
31027 Toulouse, France

M. Sassahara · J. Luciola · J. Rubira-Gerez · I. Alassane-Kpembi ·
I. P. Oswald
INP, UMR1331, Toxalim Research Centre in Food Toxicology,
Université de Toulouse, 31000 Toulouse, France

F. Lyazhri
ENVIT, INP, Université de Toulouse, 31000 Toulouse, France

C. Borin
ENFA, INP, Université de Toulouse, 31326 Castanet Tolosan,
France



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



Joelma Lucili ^{a,b,c,1}, Philippe Pinton ^{a,b,1}, Patrick Callu ^d, Joëlle Laffitte ^{a,b}, François Grosjean ^d, Martine Kolf-Clauw ^{b,e}, Isabelle P. Oswald ^{a,b}, Ana Paula F.R.L. Bracarense ^{c,*}

^a INRA, UMR 1331, ToxAlim, Research Centre in Food Toxicology, F 31027 Toulouse, France

^b Université de Toulouse, INP, UMR 1331, ToxAlim, F 31000 Toulouse, France

^c Universidade Estadual de Londrina, Laboratory of Animal Pathology, Londrina, PR, Brazil

^d Arvalis Institut du Végétal, Pouline, 41100 Villerable, France

^e Université de Toulouse, École Nationale Vétérinaire, ENVT-INP, F 31076 Toulouse, France

ARTICLE INFO

Article history:

Received 25 September 2012

Received in revised form 25 December 2012

Accepted 30 January 2013

Available online 10 February 2013

Keywords:

Trichothecenes

Mycotoxins

Pigs

MAPK kinase

Intestine

ABSTRACT

Trichothecenes induce changes in the intestinal barrier function through decreased expression of cell junction proteins and apoptosis of enterocytes. The mitogen activated protein kinases (MAPK) play an important role in the signaling pathways of cell turnover and differentiation. Using *ex vivo* and *in vivo* approaches, the purpose of this study was to investigate the ability of low doses of DON to induce histological changes in the intestine and to activate the MAPK ERK 1/2, p38 and JNK. Twelve weaning piglets received during four weeks a control diet or a DON-contaminated diet (2.3 mg DON/kg feed). Six weaning piglets were used to prepare jejunal explants (*ex vivo* model). Explants were exposed during 4 h to vehicle, 5 or 10 μ M DON. Intestinal changes were graded using a histological score. Pigs fed a DON-diet and explants exposed to DON showed a significant decrease in the jejunal score. In both models, the toxin significantly enhanced phosphorylation of ERK 1/2 and p38, whereas the increased phosphorylation of JNK was non significant. Taken together these results indicate that *in vivo* or *ex vivo* exposure of intestinal tissue to DON lead to similar intestinal lesions and activation of MAPK. These effects could impair the homeostasis of intestinal tissue in the aspects of barrier function and immune protection. The similarity of the *in vivo* and *ex vivo* results provides also strong evidence that the jejunal explant model is a good alternative for toxicological studies in intestinal tissue.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

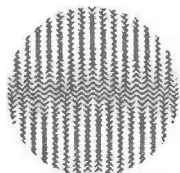
Mycotoxins are secondary metabolites produced by fungi and detected in various food commodities from many parts of the world. They are presently considered as one of the most hazardous contaminants of concern in food and feed, contaminating 25% of the world's crops each year (CAST, 2003).

The trichothecene deoxynivalenol (DON) contaminates cereals worldwide after grain infestation by *Fusarium*

* Corresponding author. Rodovia Celso Garcia Cid, PR 445, Km 380, Campus Universitário, Cx. Postal 6001, CEP 86051-980, Londrina, PR, Brazil. Tel.: +55 43 33714766; fax: +55 43 33714485.

E-mail addresses: ana.bracarense@pq.cnpq.br, anapaula@uel.br (A.P.F. R.L. Bracarense).

¹ These authors equally contributed to this work.



INRA

CENTRE DE RECHERCHES DE TOULOUSE

**LABORATOIRE DE PHARMACOLOGIE
TOXICOLOGIE (UR 66)**

Tél. 33 (0)5 61 28 51 41 (51 60)

Fax 33 (0)5 61 28 53 10

Etablissement public à caractère
scientifique et technique
placé sous la tutelle conjointe des ministres
chargés de la recherche et de l'agriculture

A CAPES

Toulouse le 1 Décembre 2010

A qui de droit,

Par la présente lettre, je confirme que dans le cadre de son doctorat, Joelma Lucioli a effectué un stage de type « sandwich » de 12 mois (Décembre 2009 à Décembre 2010) dans le Laboratoire INRA de Pharmacologie-Toxicologie au sein de l'équipe d'immuno-mycotoxicologie que j'anime. Ce stage était intégré dans le projet CAPES/COFECUB n° 593/08 (Analyse du risque de multi-contamination mycotoxique liés aux champignons de type *Fusarium* : approche toxicologique chez une espèce cible et modèle).

Joelma Lucioli s'est très bien intégré dans le laboratoire. Elle a obtenu des résultats très intéressants qui feront très rapidement l'objet d'une publication.

Je reste à votre disposition pour vous fournir toutes les informations supplémentaires que vous jugeriez utiles.

Sincères salutations,

Dr. Isabelle OSWALD
Ingénieur Agronome
Directeur de Recherche INRA
Coordinateur français du projet
CAPES/COFECUB 593/08

Institut National de la Recherche Agronomique

LABORATOIRE DE PHARMACOLOGIE - TOXICOLOGIE - 180 CHEMIN DE TOURNEFEUILLE - BP 3 -
31931 TOULOUSE CEDEX 9 - FRANCE

DECLARAÇÃO

Declaramos para os devidos fins que, Joelma Lucioli, é aluna regular do Programa de Pós-Graduação em Ciência Animal da Universidade do Estado de Santa Catarina – UDESC, CPF: 988.627.239-20, em nível de Pós-Doutorado, com projeto de pesquisa intitulado: “Uso de explantes como método complementar de análise in vitro nas pesquisas com aditivos alimentares in vivo em suínos”, com período de participação de 01/09/2024 a 31/07/2025.

É o que nos cumpre declarar.

Lages, 4 de julho de 2025.

Prof. HENRIQUE M. N. RIBEIRO FILHO
Coordenador do PPG em Ciência Animal
CAV/UDESC



Assinaturas do documento



Código para verificação: **K95E4F1I**

Este documento foi assinado digitalmente pelos seguintes signatários nas datas indicadas:



HENRIQUE MENDONCA NUNES RIBEIRO FILHO (CPF: 504.XXX.900-XX) em 04/07/2025 às 09:49:23

Emitido por: "SGP-e", emitido em 30/03/2018 - 12:42:16 e válido até 30/03/2118 - 12:42:16.

(Assinatura do sistema)

Para verificar a autenticidade desta cópia, acesse o link <https://portal.sgpe.sea.sc.gov.br/portal-externo/conferencia-documento/VURFU0NfMTlwMjJfMDAwMjQ0NjBfMjQ0NzhfMjAyNV9LOTVFNEYxSQ==> ou o site

<https://portal.sgpe.sea.sc.gov.br/portal-externo> e informe o processo **UDESC 00024460/2025** e o código **K95E4F1I** ou aponte a câmera para o QR Code presente nesta página para realizar a conferência.



CERTIFICADO

Certificamos que **JOELMA LUCIOLI** participou do XII Encontro Nacional de Diagnóstico Veterinário em Porto Alegre, durante o período de 15/10/2024 a 17/10/2024, com carga horária de 30 hora(s).

A handwritten signature in blue ink, reading 'David Driemeier'.

David Driemeier
Presidente do XII ENDIVET

A handwritten signature in blue ink, reading 'Welden Panziera'.

Welden Panziera
Presidente da comissão
científica do XII ENDIVET



Organização

Evento: **XII Encontro Nacional de Diagnóstico Veterinário**

Local: **Porto Alegre**

Participante: **Joelma Lucioli**

Data: **15/10/2024 - 17/10/2024**

Programação:

15/10/2024 - 09:00 - Abertura	15/10/2024 - 10:15 - Produção e divulgação científica Palestrante: Leonardo José Gil Barcellos
15/10/2024 - 11:20 - E-pôster	15/10/2024 - 11:30 - Almoço
15/10/2024 - 13:20 - Lançamento Livro "Diagnóstico patológico de abortamentos e mortalidade neonatal [...]	15/10/2024 - 13:30 - Encefalomielite equina Palestrante: Carolina Matto Romero
15/10/2024 - 14:45 - Patologia Veterinária Digital Palestrante: Ingeborg Maria Langohr	15/10/2024 - 16:00 - Coffee Break
15/10/2024 - 16:30 - Mulheres na ciência Palestrante: Marcia Cristina Bernardes Barbosa	16/10/2024 - 08:30 - E-pôster
16/10/2024 - 08:30 - Técnicas de detecção antigênica direta: ISH e RNAScope Palestrante: Ingeborg Maria Langohr	16/10/2024 - 09:30 - Coffee Break
16/10/2024 - 10:15 - Doenças carenciais e Intoxicações por Minerais Palestrante: Franklin Riet Correa Amaral	16/10/2024 - 11:30 - Almoço
16/10/2024 - 13:30 - Doenças entéricas de suínos Palestrante: Roberto Maurício Carvalho Guedes	16/10/2024 - 14:45 - Sequenciamento de alto desempenho para descoberta de novos agentes virais e resolução de surtos Palestrante: Fernando Rosado Spilki
16/10/2024 - 16:00 - Coffee Break	16/10/2024 - 16:30 - Patologia de aves: da necropsia ao diagnóstico molecular Palestrante: Renata Assis Casagrande
17/10/2024 - 08:30 - Doenças genéticas em animais de produção Palestrante: Alexandre Secorun Borges	17/10/2024 - 08:30 - E-pôster
17/10/2024 - 09:30 - Coffee Break	17/10/2024 - 10:15 - Patologia de animais marinhos Palestrante: Luciana Sonne
17/10/2024 - 11:30 - Almoço	17/10/2024 - 13:30 - Apresentação oral dos melhores resumos

DAVIS-THOMPSON FOUNDATION

This certificate is given to

Joelma Luciola

State:_____ License Number:_____

for completing 22.0 hours of continuing education at the following symposium:

2023 General Pathology Review Course
30 January to 3 February 2023



Francisco A. Uzal, DVM, MSc, PhD, DACVP

Chief executive officer,
Davis-Thompson Foundation



Jey Koehler, DVM, PhD, DACVP

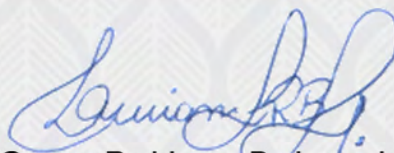
President,
Davis-Thompson Foundation

CERTIFICADO

A Escola Nacional de Gestão Agropecuária (ENAGRO) certifica que **JOELMA LUCIOLI**, portador do CPF nº *****.627.239-****, participou do curso **Módulo de noções em encefalopatias espongiformes transmissíveis - PNCEBT**, realizado na Escola de Veterinária e Zootecnia da Universidade Federal de Goiás, no período de 19 a 20 de outubro de 2022, com carga horária de 16 horas.

Goiânia/GO, 19 de outubro de 2022

Código de verificação: 63532eb9-4518-4d08-af61-62adc0a80343



Luciana Gomes Rodrigues Barbosa dos Santos
Coordenadora-Geral da Escola Nacional de
Gestão Agropecuária - ENAGRO

Número do Processo SEI:21000.071599/2022-50



CONTEÚDO PROGRAMÁTICO

- Programa Nacional de Controle da Raiva em Herbívoros e legislação (PNCRH);
- Programa Nacional de Prevenção e Vigilância da EEB e legislação (PNEEB);
- Vigilância das síndromes neurológicas no Brasil: responsabilidades compartilhadas;
- Identificação de animais com sinais nervosos;
- Procedimentos para coleta de amostras em ruminantes domésticos e EPIs;
- Técnicas de acondicionamento, meios e fluxos para envio de amostras;
- Sistema de informação: e-Sisbravet (notificação, fluxo e fichas de registro);
- Preenchimento de fichas de investigação e envio de amostras: Formulário de envio de amostras (Form-SN);
- Orientações sobre a aula prática para coleta de amostras;
- Procedimentos para coleta de amostras para diagnósticos e testes diferenciais de síndromes neurológicas em ruminantes;
- Acondicionamento e envio de amostras.



Luciana Gomes Rodrigues Barbosa dos Santos
Coordenadora-Geral da Escola Nacional de
Gestão Agropecuária - ENAGRO



DAVIS-THOMPSON FOUNDATION

This certificate is given to

Joelma Luciola

State:____ License Number:_____

for completing 1.5 hours of continuing education at the following symposium:

Rewind Friday: Gross Photography Tips
15 July 2022



Francisco A. Uzal, DVM, MSc, PhD, DACVP
Chief executive officer,
Davis-Thompson Foundation



Jey Koehler, DVM, PhD, DACVP
President,
Davis-Thompson Foundation

DAVIS-THOMPSON FOUNDATION

This certificate is given to

Joelma Luciola

State: _____ License Number: _____

for completing 20 hours of continuing education at the following symposium:

Davis-Thompson Foundation and Arab Association of Veterinary Pathologists (AAVP)

First Regional Virtual Gross Seminar of Animal Diseases

17 - 20 June 2021



Francisco A. Uzal, DVM, MSc, PhD, DACVP

Chief executive officer,
Davis-Thompson Foundation



Jey Koehler, DVM, PhD, DACVP

President,
Davis-Thompson Foundation



CERTIFICADO

Certificamos que

JOELMA LUCIOLI

Participou na qualidade de participante do 6º Congresso Brasileiro de Patologia Veterinária e XX Encontro Nacional de Patologia Veterinária [CBPV/ENAPAVE], promovido pela Associação Brasileira de Patologia Veterinária - ABPV, entre os dias 26 e 30 de julho de 2021, em formato remoto, com carga horária de 24 horas.

BRENO SOUZA SALGADO
Presidente da ABPV

Código de verificação: **7fxSdVFWXu09**

Para validar, acesse: <https://eventos.congresse.me/certificados/validar-certificado>



6º CONGRESSO BRASILEIRO DE PATOLOGIA VETERINÁRIA E XX ENCONTRO NACIONAL DE PATOLOGIA

PROGRAMAÇÃO

26/07/21 18:00 - PALESTRA ÂNCORA: MICROSCOPIA DIGITAL: A NOVA REALIDADE DO PATOLOGISTA PÓS PANDEMIA
26/07/21 08:00 - PALESTRA MAGNA | ABERTURA (clique na imagem)
26/07/21 08:00 - PALESTRA MAGNA | ABERTURA (Inglês) (clique na imagem)
27/07/21 09:00 - CINQUANA DOS GRUPOS DE ESTUDO
27/07/21 18:00 - PALESTRA ÂNCORA: INTERNATIONAL GUIDELINES FOR VETERINARY TUMOR PATHOLOGY
27/07/21 17:50 - PALESTRA ÂNCORA: INTERNATIONAL GUIDELINES FOR VETERINARY TUMOR PATHOLOGY (Tradução)
28/07/21 18:00 - PALESTRA ÂNCORA: SESSÃO CONJUNTA COM O LATIN COMPARATIVE PATHOLOGY GROUP
28/07/21 08:15 - SESSÃO ONLINE DOS MELHORES RESUMOS
29/07/21 08:30 - FÓRUM DE ENSINO: O ENSINO DE PATOLOGIA NO CONTEXTO DA PANDEMIA
29/07/21 17:55 - PALESTRA ÂNCORA: PATHOLOGY OF LABORATORY ANIMALS
29/07/21 17:55 - PALESTRA ÂNCORA: PATHOLOGY OF LABORATORY ANIMALS (Tradução)
30/07/21 09:00 - FÓRUM DE LÂMINAS DOS RESIDENTES
30/07/21 08:00 - SESSÃO SOLENE DE ENTREGA DAS PREMIAÇÕES

DIA 26/07 (09:00 - 11:00) | MINI-CURSO: PATOLOGIA ORAL (Inglês) **(Programação Premium)**
DIA 26/07 (09:00 - 13:00) | MINI-CURSO: PATOLOGIA DE EQUINOS **(Programação Premium)**
DIA 26/07 (09:00 - 13:00) | MINI-CURSO: PATOLOGIA DE SILVESTRES **(Programação Premium)**
DIA 26/07 (09:00 - 13:00) | MINI-CURSO: PATOLOGIA FORENSE **(Programação Premium)**
DIA 26/07 (09:00 - 13:00) | MINI-CURSO: PATOLOGIA ORAL **(Programação Premium)**
DIA 26/07 (14:00 - 16:00) | MINI-CURSO: PATOLOGIA DE EQUINOS (Inglês) **(Programação Premium)**
DIA 26/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE EQUINOS **(Programação Premium)**
DIA 26/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE SILVESTRES **(Programação Premium)**
DIA 26/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA FORENSE **(Programação Premium)**
DIA 26/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA ORAL **(Programação Premium)**
DIA 27/07 (14:00 - 16:00) | MINI-CURSO: PATOLOGIA DE SUÍNOS (Inglês) **(Programação Premium)**
DIA 27/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE AVES **(Programação Premium)**
DIA 27/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE DOENÇAS INFECCIOSAS **(Programação Premium)**
DIA 27/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE SUÍNOS **(Programação Premium)**
DIA 27/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE TUMORES **(Programação Premium)**
DIA 27/07 (16:00 - 18:00) | MINI-CURSO: PATOLOGIA DE TUMORES (Inglês) **(Programação Premium)**
DIA 28/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE AVES **(Programação Premium)**
DIA 28/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE DOENÇAS INFECCIOSAS **(Programação Premium)**
DIA 28/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE SUÍNOS **(Programação Premium)**
DIA 28/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE TUMORES **(Programação Premium)**
DIA 28/07 (16:00 - 18:00) | MINI-CURSO: PATOLOGIA DE DOENÇAS INFECCIOSAS **(Programação Premium)**
DIA 29/07 (14:00 - 18:00) | MINI-CURSO: CITOPATOLOGIA **(Programação Premium)**
DIA 29/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE ANIMAIS AQUÁTICOS **(Programação Premium)**
DIA 29/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE BOVINOS **(Programação Premium)**
DIA 29/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA MOLECULAR **(Programação Premium)**
DIA 29/07 (16:00 - 17:00) | MINI-CURSO: PATOLOGIA MOLECULAR (Tradução) **(Programação Premium)**
DIA 29/07 (17:00 - 18:00) | MINI-CURSO: PATOLOGIA DE ANIMAIS AQUÁTICOS (Inglês) **(Programação Premium)**
DIA 30/07 (14:00 - 18:00) | MINI-CURSO: CITOPATOLOGIA **(Programação Premium)**
DIA 30/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE ANIMAIS AQUÁTICOS **(Programação Premium)**
DIA 30/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE ANIMAIS AQUÁTICOS (Inglês) **(Programação Premium)**
DIA 30/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA DE BOVINOS **(Programação Premium)**
DIA 30/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA MOLECULAR **(Programação Premium)**
DIA 30/07 (14:00 - 18:00) | MINI-CURSO: PATOLOGIA MOLECULAR (Tradução) **(Programação Premium)**

DAVIS-THOMPSON FOUNDATION

This certificate is given to

Joelma Luciola

State:____ License Number:_____

for completing 1.5 hours of continuing education at the following symposium:

Patologia de Doenças Micóticas
9 November 2022



Francisco A. Uzal, DVM, MSc, PhD, DACVP

Chief executive officer,
Davis-Thompson Foundation



Jey Koehler, DVM, PhD, DACVP

President,
Davis-Thompson Foundation

DAVIS-THOMPSON FOUNDATION

This certificate is given to

Joelma Luciola

State: _____ License Number: _____

for completing 1.5 hours of continuing education at the following symposium:

Plantas Tóxicas Para Animais De Produção No Brasil

18 May 2022

Breno S. Salgado

Breno Salgado, DVM, MSc, PhD

Presidente da Associação Brasileira de
Patologia Veterinária



Jey Koehler

Jey Koehler, DVM, PhD, DACVP

President,
Davis-Thompson Foundation

DAVIS-THOMPSON FOUNDATION

This certificate is given to

Joelma Luciola

State:____ License Number:_____

for completing 1.0 hour of continuing education at the following symposium:

Serie de Seminarios de Histopatología del LCPG:
Enfermedades del Aparato Respiratorio
15 September 2022



Francisco A. Uzal, DVM, MSc, PhD, DACVP

Chief executive officer,
Davis-Thompson Foundation



Jey Koehler, DVM, PhD, DACVP

President,
Davis-Thompson Foundation

WORKSHOP

PATOLOGIA VETERINÁRIA:

DE NORTE A SUL, DE LESTE A OESTE



CERTIFICADO

Certificamos que **JOELMA LUCIOLI** participou na qualidade de OUVINTE do **Workshop - Patologia Veterinária: de Norte a Sul, de Leste a Oeste**, promovido pela Associação Brasileira de Patologia Veterinária - ABPV, realizado nos dias 08, 15, 22 e 29 de setembro e dia 06 de outubro de 2022, em formato remoto, com carga horária de 10 horas.

Ricardo Barbosa de Lucena
Presidente da ABPV

Francisco de Assis Leite Souza
Diretor Científico da ABPV



f [abpv.vet.br](https://www.abpv.vet.br)

DAVIS-THOMPSON DVM FOUNDATION

This certificate is given to

JOELMA LUCIOLI

State: _____ License Number: _____

for completing **2** hours of continuing education at the following symposium:

PATOLOGIA SUÍNA

10 Março 2021

Breno S. Salgado

BRENO SALGADO, DVM, MSc,
PhD

Presidente da Associação Brasileira
de Patologia Veterinária (ABPV)



Francisco A. Uzal

Francisco A. Uzal, DVM,
MSc, PhD, DACVP

CEO, Davis-Thompson Foundation

DAVIS-THOMPSON FOUNDATION

CERTIFICATE OF ATTENDANCE

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Joelma Luciola

WHO COMPLETED 8 CONTACT HOURS OF (NON-MEDICAL) VETERINARY
CONTINUING EDUCATION AT THE FOLLOWING SYMPOSIUM:

20TH DAVIS-THOMPSON DIAGNOSTIC PATHOLOGY SYMPOSIUM

“GLOBAL ANIMAL HEALTH AND WORKING IN DEVELOPMENT”

IN CONJUNCTION WITH THE 63RD ANNUAL AAVLD ANNUAL MEETING

15 OCTOBER 2020

This course meets the requirements for non-medical 8 hours of continuing education credit for veterinarians in subject matter category two (interactive-distance) using the delivery method of seminar/lecture in jurisdictions which recognize AAVSB's RACE approval; however, participants should be aware that some boards have limitations on the number of hours accepted in certain categories and/or restrictions on certain methods of delivery of continuing education.

Dr. James Britt
(501) 912-1449

AAVSB RACE Provider No: 149
RACE Program # 149-44289



FRANCISCO A. UZAL, DVM, MSc, PhD, DACVP
CEO, DAVIS-THOMPSON FOUNDATION

No 149-44289