

# Probiotic and prebiotics: impact on bioavailability of aflatoxin B<sub>1</sub> in milk

Rute Vasconcelos<sup>1</sup>, Katia Wochner<sup>2</sup>, Tânia Becker-Algeri<sup>3</sup>, Paula Rodrigues<sup>4,\*</sup>, Deisy Drunkler<sup>5,\*</sup>

<sup>1</sup>ESA, Polytechnic Institute of Bragança, Portugal / Universidade Tecnológica Federal do Paraná (UTFPR), Medianeira, Brasil (Programa de Dupla Diplomação com Mestrado em Qualidade e Segurança Alimentar, ESA/IPB); <sup>2</sup> Programa de Pós Graduação em Tecnologia de Alimentos (PPGTA)/ UTFPR, Medianeira, Paraná, Brasil; <sup>3</sup> Universidade Federal do Rio Grande (FURG), Rio Grande do Sul, Brasil; <sup>4</sup> CIMO/ESA, Instituto Politécnico de Bragança, Portugal; <sup>5</sup> PPGTA/ UTFPR, Medianeira, Paraná, Brasil; <sup>3</sup>Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal <sup>4</sup>Programa de Pós-Graduação em Tecnologia de Alimentos (PPGTA), UTFPR, Medianeira, PR, Brasil.

\* prodrigues@ipb.pt, deisydrunkler@utfpr.edu.br

## Introduction

Milk represents one of the most important dietary sources of nutrients for humans due to its rich nutritional composition. However, milk consumption is also potentially related to the ingestion of chemical contaminants such as mycotoxins. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a highly toxic mycotoxin, for both animals and humans. This toxin is frequently detected in milk, and it results from the ingestion of contaminated feed by producing animals. Given its high toxicity, methods of AFB<sub>1</sub> decontamination, including the use of probiotic microorganisms, have been tested in milk. But the total amount of ingested contaminant does not always reflect the amount available for absorption (bioavailability), so *in vitro* digestion models are used to determine human oral bioavailability of food contaminants. Regarding prebiotics, few studies have evaluated the decontamination of action of these compounds and it is suggested further that the probiotics to be synergistic in the removal of mycotoxins.

## Materials and methods

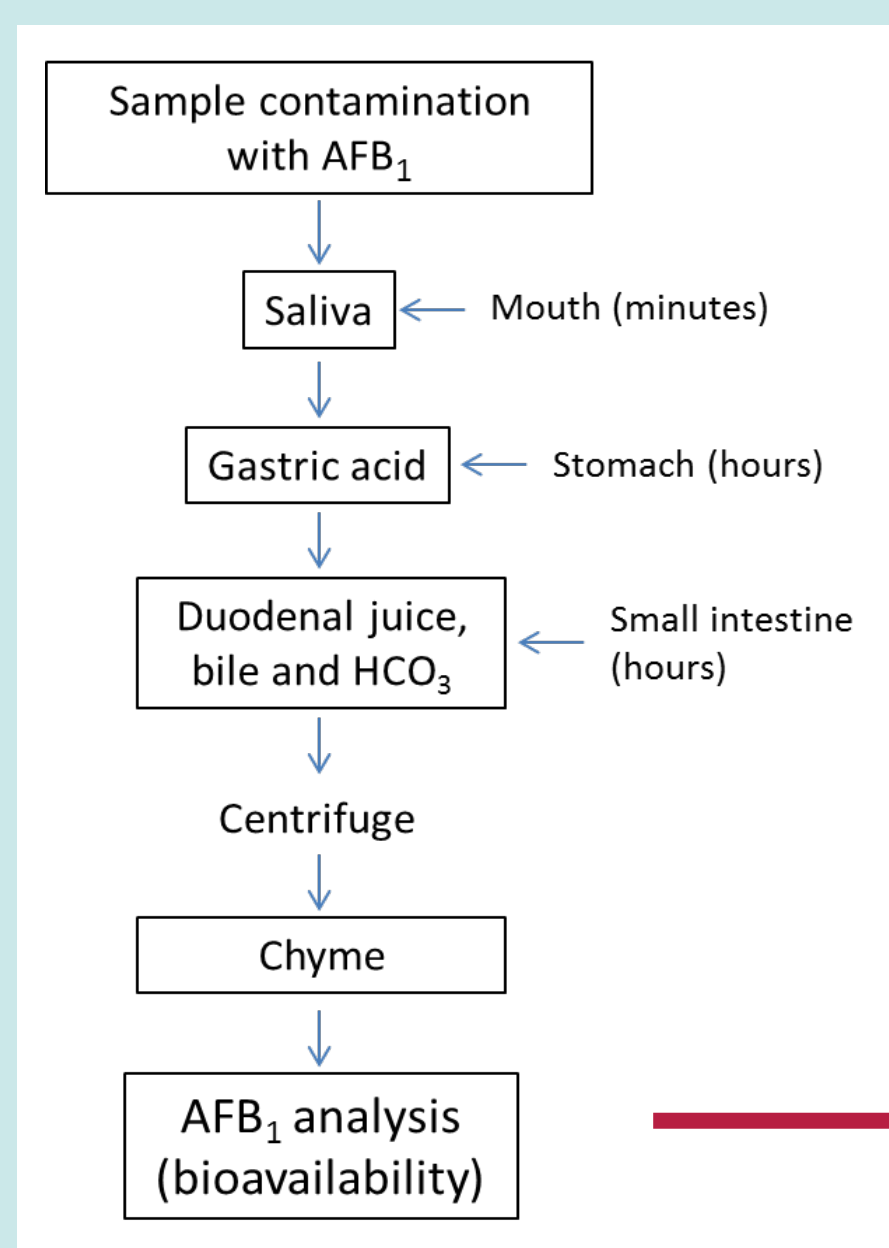
Milk samples spiked with AFB<sub>1</sub> (10 µg.L<sup>-1</sup>) were inoculated with *L. plantarum* and with the prebiotic compounds inulin, beta-glucan, polydextrose and oligofructose, isolated and combined, in a total of 12 different assays plus two controls (Table 1). The samples were subjected to *in vitro* digestibility (Figure 1). AFB<sub>1</sub> was quantified in samples by UHPLC-FLD before and after digestion.

**Table 1** - Matrix planning Plackett Burman of 12 trials with 02 central points.

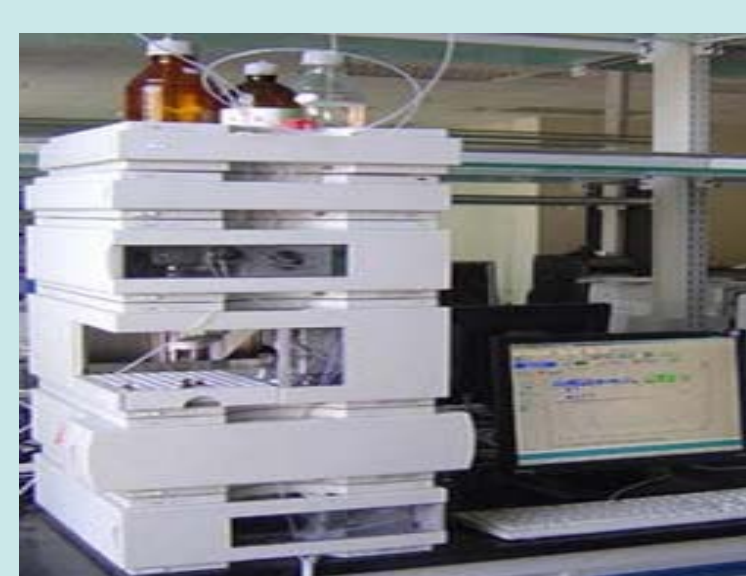
| Test | x <sub>1</sub> <sup>a</sup> | x <sub>2</sub> <sup>b</sup> | x <sub>3</sub> <sup>c</sup> | x <sub>4</sub> <sup>d</sup> | x <sub>5</sub> <sup>e</sup> | x <sub>6</sub> <sup>f</sup> |
|------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 1    | +1                          | -1                          | +1                          | -1                          | -1                          | -1                          |
| 2    | +1                          | +1                          | -1                          | +1                          | -1                          | -1                          |
| 3    | -1                          | +1                          | +1                          | -1                          | +1                          | -1                          |
| 4    | +1                          | -1                          | +1                          | +1                          | -1                          | +1                          |
| 5    | +1                          | +1                          | -1                          | +1                          | +1                          | -1                          |
| 6    | +1                          | +1                          | +1                          | -1                          | +1                          | +1                          |
| 7    | -1                          | +1                          | +1                          | +1                          | -1                          | +1                          |
| 8    | -1                          | -1                          | +1                          | +1                          | +1                          | -1                          |
| 9    | -1                          | -1                          | -1                          | +1                          | +1                          | +1                          |
| 10   | +1                          | -1                          | -1                          | -1                          | +1                          | +1                          |
| 11   | -1                          | +1                          | -1                          | -1                          | -1                          | +1                          |
| 12   | -1                          | -1                          | -1                          | -1                          | -1                          | -1                          |
| 13   | 0                           | 0                           | 0                           | 0                           | 0                           | 0                           |
| 14   | 0                           | 0                           | 0                           | 0                           | 0                           | 0                           |
| CP*  | 0                           | -                           | -                           | -                           | -                           | -                           |
| CN** | -                           | -                           | -                           | -                           | -                           | -                           |

<sup>a</sup> AFB<sub>1</sub> concentration (µg.L<sup>-1</sup>);  
<sup>b</sup> Time (h);  
<sup>c</sup> Inulin;  
<sup>d</sup> Oligofructose;  
<sup>e</sup> B-Glucan;  
<sup>f</sup> Polydextrose  
 • Positive control;  
 • \*\* Negative control

**Levels:**  
 [AFB<sub>1</sub>]: +1 / 0 / -1 = 10 / 7.5 / 5  
 Time: +1/ 0 / -1 = 6h, 3h, 0h;  
 % Prebiotics: +1/0/-1 = 0.75/0.38/0



**Figure 1** – Scheme of *in vitro* digestion and detection and quantification of available AFB<sub>1</sub> after the various treatments



## Objective

The study aimed to evaluate the effect of the probiotic microorganism *Lactobacillus plantarum* BG112 and of several prebiotics (inulin, beta-glucan, polydextrose and oligofructose), alone or combined, in the bioavailability of AFB<sub>1</sub> in milk artificially contaminated with the mycotoxin, after digestibility in an *in vitro* digestion model.

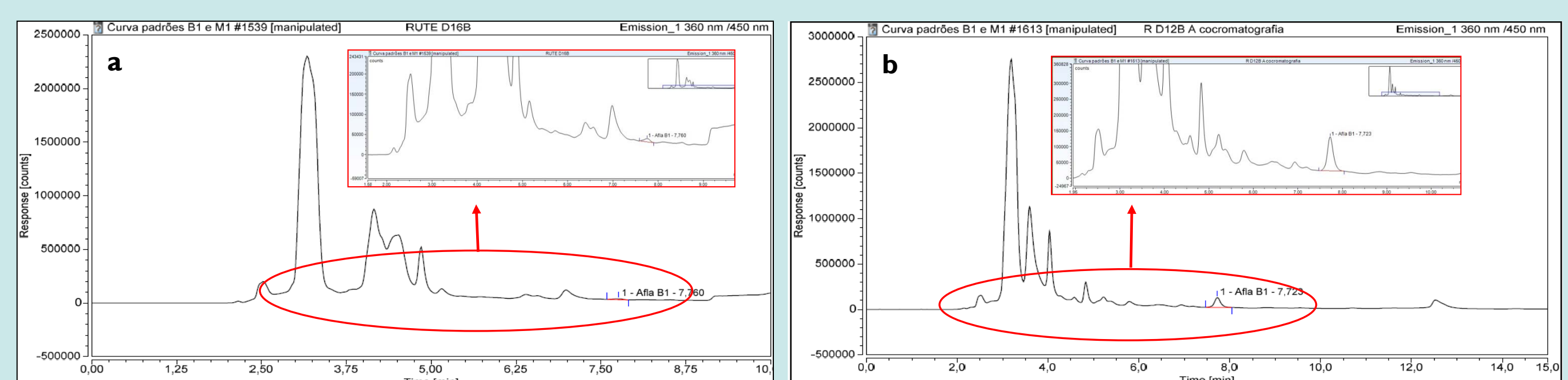
## Results and Discussion

- The bioavailability of AFB<sub>1</sub> on milk obtained for the various probiotic and prebiotics combinations after *in vitro* digestion ranged from 15.6 to 35.6% of the spiked amount.
- The addition of *L. plantarum* BG112 alone on AFB<sub>1</sub> contaminated milk resulted on AFB<sub>1</sub> bioavailability of 27.1 ± 0.93%, a level similar to that reported by other authors, emphasizing the binding capacity of the acid lactic bacteria with AFB<sub>1</sub>.
- Concerning prebiotics effect, bioavailability of AFB<sub>1</sub> varied between 15 and 51%. The best result was observed for prebiotics inulin and oligofructose when tested individually.

**Table 2** – Bioavailability of AFB<sub>1</sub> after *in vitro* digestion with the various probiotic and prebiotics combinations.

| Test | % Bioavailability |
|------|-------------------|
| 1    | 15,92±0,79        |
| 2    | 15,62±0,55        |
| 3    | 26,53±2,13        |
| 4    | 21,25±1,60        |
| 5    | 20,00±2,00        |
| 6    | 30,76±1,74        |
| 7    | 35,63±0,28        |
| 8    | 51,09±0,99        |
| 9    | 39,34±0,77        |
| 10   | 26,78±0,81        |
| 11   | 30,55±0,74        |
| 12   | 27,12±0,93        |
| 13   | 28,01±0,27        |
| 14   | 21,00±0,30        |
| CP*  | 101±6,3           |
| CN** | ND                |

**Figure 2** - Chromatograms of milk samples fortified with AFB<sub>1</sub>, after *in vitro* digestion: a) without detectable AFB<sub>1</sub>; b) with AFB<sub>1</sub>.



## Conclusions

- Bioavailability of AFB<sub>1</sub> was reduced by the addition of *L. plantarum* and prebiotics under all tested conditions.
- *L. plantarum* can be incorporated into a food matrix in order to reduce the bioavailability of AFB<sub>1</sub>.
- The results help explain how some natural components present in food can help modulate the intake of certain toxic compounds.