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## **Modification of aflatoxin B1 and ochratoxin A toxicokinetics in rats administered a yeast cell wall preparation**

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**Modification of aflatoxin B1 and ochratoxin A toxicokinetics in rats administered a yeast cell wall preparation**

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6 **2 Modification of aflatoxin B<sub>1</sub> and ochratoxin A toxicokinetics in rats**  
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8 **administered a yeast cell wall preparation**  
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*A yeast product alters mycotoxins toxicokinetics***Abstract**

2 The cell wall of *Saccharomyces cerevisiae* can bind mycotoxins *in vitro* but there is scarce  
6 information on whether this property decreases the absorption of mycotoxins *in vivo*. The  
8 effect of a yeast cell wall preparation (YCW) on toxicokinetics and balance excretion (urine  
10 and faeces) of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and ochratoxin A (OTA) was tested in rats after oral  
12 administration of each toxin. The <sup>3</sup>H-labelled mycotoxins were used at low doses. Co-  
14 administration of YCW with AFB<sub>1</sub> decreased the extent, but not the rate of absorption.  
16 Concurrently, radioactivity excreted in faeces increased by up to 55% when compared to  
18 controls, whilst the excretion in urine decreased ( $p < 0.05$ ). The effect of YCW on OTA was  
20 less marked, although it increased radioactivity excretion in faeces (up to 16%;  $p < 0.05$ ) it  
22 did not result in changes in urine and toxicokinetic parameters. The *in vivo* effect is in  
24 agreement with the reported *in vitro* binding ability for these toxins (AFB<sub>1</sub> > OTA). In  
26 conclusion, these results indicate that YCW could be used to protect monogastric animals  
28 against exposure to low dietary levels of selected mycotoxins.

30 **Keywords:** aflatoxin B<sub>1</sub>; ochratoxin A; yeast cell wall; detoxification; adsorbing agent;  
32 toxicokinetics; rat

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## Introduction

Mycotoxins are secondary fungal metabolites commonly found in foods and feeds (Placinta et al. 1999; Garon et al. 2006; Binder et al. 2007). Many of these secondary metabolites are toxic to humans and animals, affecting health, reducing animal performance and causing substantial economic losses. The application of good agricultural and storage practices can reduce contamination but some factors contributing to fungal development, such as climatic conditions, are beyond human control and the absence of mycotoxins in the ration of farm animals cannot be fully assured. The presence of mycotoxins in feeds is of concern in livestock production as it poses a risk for animals and also for consumers if toxic molecules are transferred into animal products. In most countries contamination levels of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in feeds are regulated and thus, highly-contaminated feeds are not normally given to animals. However, contamination levels just below the legal limit may still have a negative, long-term impact on production. In addition, on-farm produced feeds are seldom controlled, which may result in higher than tolerable mycotoxin ingestion.

Several methods have been investigated for their capacity to remove, or reduce, mycotoxins in contaminated feeds (reviewed by Jouany 2007). Some physical and chemical treatments such as the use of ammonia are expensive or simply not adapted to the treatment of feeds destined for livestock (Niderkorn et al. 2007). The use of products, which could be defined as mycotoxins inactivators, to reduce absorption of mycotoxins in the gastrointestinal tract of animals, is one such practical alternative which is currently receiving increased attention. Among these products, organic adsorbents such as yeast cell wall (YCW) have been shown to rapidly form complexes in vitro with mycotoxins (Devegowda et al. 1998). Feeds multicontaminated by mycotoxins are frequent in the field (Scudamore et al. 1998; Mansfield et al. 2008) and the ability of YCW to bind several mycotoxins makes them suitable for use under practical feeding conditions. In vivo studies have shown that YCW can reduce the adverse effects of mycotoxins in different animal species; swine (Swamy et al. 2002), horse (Raymond et al. 2003), laying hens (Chowdhury et al. 2005), and dairy cows (Korosteleva et al. 2007). In these studies the detoxification efficiency of YCW has been mainly evaluated on the basis of animal performance and/or the absence of toxicity, but the precise mechanism responsible for the beneficial effect of this additive has not yet been clearly demonstrated. In lactating dairy cows receiving AFB<sub>1</sub>, a decrease in the excretion of aflatoxin M<sub>1</sub> in milk was observed in some studies (Diaz et al. 2004; Masoero et al. 2007) but

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not in others (Stroud 2006; Waltman 2008; Kutz et al. 2009). A decrease in milk carryover suggests that mycotoxin absorption was impaired.

The purpose of this study was to evaluate the effect of a YCW-based preparation on AFB1 and ochratoxin A (OTA) toxicokinetics in rats. AFB1 and OTA were chosen as they are the two most common mycotoxins found in feeds and which can cause a variety of toxic responses in animals, including nephrotoxic, hepatotoxic, teratogenic, and carcinogenic effects. Mycotoxins were <sup>3</sup>H-labelled and radioactivity was monitored in faeces, urine and plasma following oral administration. The YCW product was tested at two different doses and mycotoxins were used at relatively low concentrations and in a single dose (< 1% LD50) to prevent overt perturbations of liver and kidney functions.

**Materials and Methods****Chemicals**

Non-radioactive AFB1 and pentobarbital sodium were purchased from Sigma Chemical (St Quentin, France). [<sup>3</sup>H]-AFB1 (two different lots were used with a specific activity of 6.9 and 17.5 Ci/mmol; 97% and 98.6% purity) and [<sup>3</sup>H]-OTA (specific activity: 5 Ci/mmol; 99.6% purity) were supplied by Hartmann Analytic (GmbH, Braunschweig, Germany) in methanol and ethanol solutions, respectively. A stock solution of non radiolabelled AFB1 was prepared in methanol at a concentration of 0.2 mg/mL. Ready Safe™ LSC cocktail was supplied by Beckman Coulter (Villepinte, France), soluene-350® by Perkin-Elmer (Courtaboeuf, France). A preparation composed of modified cell walls extracts of *Saccharomyces cerevisiae* strain 1026 was provided by Alltech Inc. (Yeast Cell Wall (YCW), purity 90%, batch No FR71535-1, Nicholasville, U.S.A.). YCW was used as a suspension in 0.9% NaCl at a concentration of 20 mg/ml. Suspensions were freshly prepared before use.

**Animals and administration of mycotoxin**

Eight week-old male Sprague-Dawley (265 ± 30 g) rats were purchased from Dépre Centre (Saint-Doulchard, France). Rats had free access to water and were fed *ad libitum* with certified 2016 Teklad Global 16% Protein Rodent Diet (Harlan Inc, WI, U.S.A.). The animal room was environmentally controlled, animals were provided with 12 hours light each day with the temperature range maintained between 22 to 25 °C, and a relative humidity range of 40-55%. Studies were conducted in accordance with the applicable national and European

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guidelines and regulations for experimentation with animals (see [http://www2.vet-lyon.fr/ens/expa/acc\\_regl.html](http://www2.vet-lyon.fr/ens/expa/acc_regl.html) for details).

Mycotoxins were administered by gavage through a gastric intubation at a constant volume of 2 ml of a dose solution per kg of BW. The exact amount administered was determined by weighing the dosing syringe before and after gavage. Feed was removed from cages 12 h before gavage and rats were fed again 6 h post gavage. The dose solution contained AFB1 or OTA (specific activity: 40  $\mu$ Ci/kg of BW) with or without YCW at one of two concentrations. Stock mycotoxin solutions were diluted to the desired dose volume with 0.9% NaCl and 0.1M NaHCO<sub>3</sub> for AFB1 and OTA, respectively. For the high dose of AFB1 (18.12  $\mu$ g/kg BW) a 10-fold isotopic dilution was prepared by co-diluting [<sup>3</sup>H]-AFB1 (specific activity: 6.9 Ci/mmol) with non radiolabelled AFB1. The YCW suspension was replaced by a solution of 0.9% NaCl in control animals.

### ***Study design***

A first assay using AFB1 was performed to optimize the protocol. In this trial a comparison between the use of blood and plasma samples for monitoring absorption was made. Radioactivity in urine and faeces was monitored for up to 15 days to evaluate the minimum collection time necessary to correctly assess excretion. Two groups of rats were included, each group was made up of 3 lots that received a single dose of AFB1 (40  $\mu$ Ci and 18.12  $\mu$ g/kg BW) alone (lot 1) or in combination with YCW at 4 mg/kg BW (lot 2) and 10 mg/kg BW (lot 3). In group 1, 12 rats (3 per lot) were randomly and individually placed in metabolism cages (U.A.R., Epinay sur Orge, France). Urine and faeces were collected at 6, 12, 24, 36 and 48 h, and then every 24 h up to 15 days post dose. Following each collection cages were meticulously rinsed with water/methanol solution (1/1; v/v) and washing solutions collected in order to evaluate possible loss of radioactivity due to urine remaining within the cages. Each sample (urine, faeces and recovered washings) were weighed and stored at -20° C until analysis. Animals were sacrificed by cervical dislocation following the last collection of excreta. Liver, kidneys, jejunum, ileum and caecum of six animals (3 rats control and 3 rats YCW low dose) were dissected, weighed and aliquots were stored at -20 °C until analysis. Intestinal segments were processed without their contents. After sampling, viscera from each animal were regrouped with the carcass, homogenized and analyzed for total body radioactivity.

In group 2 (n=117; 39 rats per lot) 3 rats were taken at each time point and anaesthetised by an intraperitoneal injection of 50 mg/kg BW sodium pentobarbital. Blood (approximately

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3 mL) was collected using the vacutainer<sup>®</sup> system (Beckson Dickinson) into heparinized tubes (Beckson Dickinson) by ex-sanguination from the abdominal aortic vein at 0 (no toxin), 1, 2, 4, 5, 6, 8, 10, 14, 24, 36, 48 and 72 h post dose. After sampling rats were euthanized. An aliquot of blood was immediately used for analysis as described below. The remaining blood sample was centrifuged ( $1000 \times g$ , 5 min at 4 °C) and the plasma fraction decanted into clean tubes and stored at – 20 °C until analysis.

Based on this first study with AFB1 a good correlation between plasma and blood samples was established, only plasma samples were collected on the second AFB1 and OTA studies. In addition, in the second study urine and faeces were collected for three days, as the bulk of the radioactivity was recovered within the first 72 h post dosing.

The second AFB1 experiment was conducted using a lower dose (0.716 µg/kg BW), which was provided by [<sup>3</sup>H]-AFB1 alone (specific activity: 17.5 Ci/mmol). This dose, when extrapolated to livestock species is in line with the European Community limitation of 0.02 mg/kg feed (directive 2003/100/EC). In a similar way, doses of YCW were 16.4 and 65.7 mg/kg BW, which were within the range recommended by the YCW manufacturer for different livestock species when calculated based on their inclusion in feed. For the excretion study, 15 rats were used (5 per lot) and urine and faeces were collected at 6, 12, 24, 36, 48 and 72 h post dosing. For the absorption study (n=117; 39 rats per lot), plasma was obtained and stored as described above at 0 (no toxin), 1, 2, 4, 5, 6, 8, 10, 14, 24, 36, 48 and 72 h post dose.

The OTA study was similar to the second AFB1 study with 15 (5 per lot) and 63 rats (21 per lot) used for excretion and plasma measurements, respectively. Urine was collected for 3 days and faeces for 10 days. OTA was used at a dose of 3.22 µg/kg BW for a total radioactivity of 40 µCi per rat as described above.

***Sample preparation and analysis***

Before analysis faecal samples were homogenized in distilled water, 10% (w/w) dilution. Faecal homogenates (50 mg) were incubated at 50 °C overnight with 1 ml of soluene<sup>®</sup>-isopropanol solution (1/1; v/v) and 0.5 ml of 30% hydrogen peroxide. Blood samples (50 µl) were treated as faecal samples but were processed immediately after collection in order to minimize non-radioactive scintillation counting. All samples, i.e. blood, faecal homogenate, urine (100 µl), plasma (100 µl), and wash water from metabolic cages were mixed with 10 ml of Ready Safe<sup>™</sup> scintillation fluid and stabilized overnight in the dark before counting the



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radioactivity in a LS 1801 scintillation counter (Beckman Coulter, Villepinte, France). All samples were analyzed in triplicate, except for blood samples that were analyzed in duplicate.

Tissue radioactivity was measured by liquid scintillation counting after oxidative combustion with an oxidizer (Packard 306 Oxidizer, Packard Instruments, Meridien, CT, USA), using Permafluor E+ MonophaseS<sup>®</sup> (Packard Instruments) as the scintillation cocktail. Viscera and carcase samples (200 mg) were packed into cellulose combustion cones imbibed with 100  $\mu$ l of an organic-based combustion aid solution Combustaid<sup>®</sup> (Packard Instruments) and were analyzed in triplicate and quadruplicate, respectively.

### ***Toxicokinetics and statistical data analysis***

Non-compartmental methods were used to estimate the toxicokinetic parameters of radiolabelled mycotoxins. The parameters  $C_{max}$  and  $T_{max}$  were observed values defined as the highest concentration achieved and the required time to achieve it, respectively.  $AUC_{0-last}$  was the area under plasma concentration versus time curve, calculated by the trapezoidal method between the first and the last measurable concentration.  $AUC_{0-\infty}$  was the  $AUC_{0-last}$  extrapolated to infinity by the ratio of the last measurable concentration to the terminal slope  $k_e$ . Elimination constant ( $k_e$ ) was determined by using the Kinetica software (InnaPhase, Champ-sur-Marne, France, release 4.0).  $T_{1/2}$  corresponds to the terminal half-life determined as the ratio of Neperian logarithm of 2 to the terminal slope ( $k_e$ ). Due to the experimental design, it was not possible to conduct a statistical analysis directly on the kinetic parameters (Lellouch, Lazar 1996). Samples for toxicokinetic parameters calculation were obtained from three different rats at each sampling time. Radioactivity values measured in plasma and blood were compared at each sampling time using non-parametric, one-way-analysis of variance (Kruskal-Wallis test) using SAS version 8 (SAS Institute Inc., Cary, NC). The Kruskal-Wallis test was also used to analyse excretion data (urine and faeces). Significance was declared at the 5% probability level.

### **Results**

No overt toxic effects, sickness or behavioural anomalies were observed in any animals in any of the studies. In the first AFB1 study radioactivity values for plasma samples were about half that of blood, but profiles were similar between both matrices (data not shown). Although the recovery was lower with plasma, this matrix was used in subsequent studies because, unlike blood, plasma samples could be stored and analysed all at the same time.

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Radioactivity recovered in urine after 3 days and up to 15 days following the gavage was 1.3%, which did not represent a large proportion of the total. In faeces it represented a larger fraction of approximately 15%. In both cases, however, no differences were observed between treatments ( $p > 0.05$ ; data not shown), justifying the 72 h collection period of later studies following gavage administration.

Data from all three studies are presented in Tables 1 and 2, but results in the text for AFB1 refer to the low dose study unless otherwise stated. Toxicokinetic parameters are summarized in Table 1 with an example of plasma profiles for [ $^3\text{H}$ ]-AFB1 (Figure 1). Radioactivity from AFB1 was detected 1 h after dosing, reaching a maximum value at 4 to 5 h post dosing and decreasing slowly thereafter with an elimination half-life ( $T_{1/2}$ ) of about 64 h. The combination of YCW with [ $^3\text{H}$ ]-AFB1 altered plasma profiles, namely the absorption phase, irrespective of YCW dose. This decrease in the oral bioavailable fraction of AFB1 was not accompanied by modifications of the absorption rate.  $C_{\max}$  and AUC were lower in YCW treated lots when compared to values obtained in respective control groups. The average decrease of  $\text{AUC}_{0-\infty}$  after co-administration of YCW was approximately 38% and was independent of dose, except for high YCW at the high AFB1 concentration which resulted in a less marked decrease (Table 1). In contrast, the parameters of elimination phase ( $T_{1/2}$  and  $k_e$ ) were not influenced by YCW co-administration.

The excretion balance in the presence of YCW (Table 2) was in agreement with the changes observed in the plasma toxicokinetic parameters. Urinary excretion of radioactivity was lower after co-administration of YCW ( $p < 0.05$ ), while faecal excretion increased ( $p < 0.05$ ) (Table 2). Excretion in faeces increased by as much as 55% when compared to controls without YCW. The level of YCW inclusion did not have any effect on radioactivity excretion.

In the OTA study this toxin reached a higher maximum concentration than AFB1 in plasma, resulting in higher calculated AUC parameters (Table 1). In contrast, the YCW treatment showed a less perceptible effect during the absorption phase. In YCW treated animals OTA concentration in plasma was lower than control animals 1 h after gavage ( $p < 0.05$ ). However, beyond 1 h post-gavage OTA concentrations were the same in all three groups ( $p > 0.05$ ). Although the YCW treatment did not affect urinary excretion, it increased toxin elimination in faeces ( $p < 0.05$ ; Table 2). However, this increase was less marked than AFB1, approximately 15% compared to 55% for the latter.

Metabolic cages were rinsed and the wash water counted to address the possibility that urine was not fully collected and a proportion of urinary radioactivity was unaccounted for.

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Cage contamination was 1.3%, 3.3% and 5.2% of the total radioactivity excreted for the low-AFB1, high-AFB1 and OTA experiments, respectively (Table 2)(data not shown). Whether these values were added to the urine radioactivity or not did not affect the results. The effect of YCW on the level of radioactivity accumulating in rats was assayed using animals from the first AFB1 experiment. At 15 days post-treatment the residual radioactivity in the carcasses of the control group was  $2.5\% \pm 0.68$  (mean  $\pm$  SD) compared to  $1.6\% \pm 0.41$  for the low YCW group ( $p = 0.12$ ). Trace amounts of radioactivity (0.005%) were found in kidneys and different segments of the gastrointestinal tract. The liver accounted for the bulk of viscera radioactivity with  $0.31\% \pm 0.034$  and  $0.19 \pm 0.082$  for control and YCW group, respectively ( $p < 0.05$ ).

## Discussion

Mycotoxin sequestration in the gastrointestinal tract by adsorbing agents such as yeast cell walls could be a promising strategy to protect against the toxic effect of these feed contaminants. However, there is still scarce information on their effect in vivo. Recent reports indicate a positive effect on animal production performance and a reduction of mycotoxin carryover into milk (Diaz et al. 2004; Chowdhury et al. 2005; Korosteleva et al. 2007; Masoero et al. 2007). In contrast, the use of these kind of additives do not always result in a positive effect on the parameter measured (Stroud 2006; Waltman 2008; Kutz et al. 2009). The type of yeast could be at the origin of these differences among studies. The cell wall polysaccharide responsible for mycotoxin binding such as the alkali insoluble fraction of  $\beta$ -D-glucans (Yiannikouris et al. 2004b) varies in quality and quantity between species and strains (Nguyen et al. 1998). In addition, it has been described that for a same strain the growth conditions and the preparation process influence the adsorption capacity of yeast cell wall additives (Pradelles et al. 2008). The inconsistency between studies, however, could also be due to other reasons such as the mode of incorporation of the preparation into the diet or by the type of feed and mycotoxin present (Battacone et al. 2009; Blank, Wolfram 2009; Masoero et al. 2009). Trials involving domestic animals involve many variables which are difficult to control between studies. The lack of a positive response reported in some trials using these mycotoxin adsorbing agents has also been reported for other widely used feed additives such as probiotics or feed enzymes (Beauchemin et al. 2003; Robinson, Erasmus 2009). It is clear that more studies are needed to better understand the underlying factors inducing this variability and correctly evaluate their efficacy.

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4 The present study, using a rat animal model, shows that a preparation exclusively  
5 2 composed of yeast cell walls extracted from *Saccharomyces cerevisiae* strain 1026  
6 significantly reduces intestinal absorption of AFB1. It is worth mentioning that in the  
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9 4 toxicokinetic model used, rats were in a fasted state when the mycotoxin-YCW mixture was  
10 administered. Under natural conditions mycotoxins are contained in feeds and it is known  
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12 6 that the presence of feeds in the gastrointestinal tract may affect the bioavailability of  
13 different xenobiotic molecules (Wonnemann et al. 2006; Disanto, Golden 2009).

15 8 The toxicokinetics of AFB1 and OTA were investigated using low mycotoxin doses.  
16 These doses were calculated based on the maximum concentrations allowed or tolerated in  
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19 10 animal feeds by the European legislation (2002/32/CE and 2006/576/CE). In addition, these  
20 low doses are equivalent, in mg per kg BW, to the quantity that could be ingested by farm  
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23 12 animals under normal production conditions. Although these mycotoxins have never been  
24 investigated in rats at such low oral levels blood and/or plasma kinetics of both toxins were in  
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26 14 agreement with previous reports. Galtier *et al.*, (1979) and Coulombe and Sharma (1985)  
27 observed a biological half life of 55 h for OTA and 91.8 h for AFB1 (vs 63-65 h and 50-65h  
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29 16 in the present study) following the oral administration of unlabeled OTA 2.5 mg kg<sup>-1</sup> BW and  
30 [3H]-AFB1 600 µg kg<sup>-1</sup> BW, respectively. The peak of radioactivity in blood was reached  
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32 18 between 1 and 4 h following unlabeled OTA oral administration (Suzuki et al. 1977) and that  
33 of <sup>14</sup>C-AFB1 in plasma was reached 1 h after the same mode of administration (Wong, Hsieh  
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35 20 1978). Discrepancies with literature data were minor and may be due to differences in  
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39 protocol design.

40 22 In the current study results from toxicokinetic parameters indicate that the YCW  
41 treatment modified the absorption phase. The AUC decreased in the YCW treated groups  
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44 24 whereas the  $K_e$  was similar in all groups. In addition, the increase in cumulative radioactivity  
45 in faecal material was associated with a decrease in cumulative urinary excretion, mainly for  
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48 26 AFB1 treatments. At the end of the experiment, up to 64% and 51% of total radioactivity was  
49 recovered in urine and faeces for AFB1 and OTA, respectively. This amount of recovery is  
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51 28 normally found with tritium labelled molecules administered at very low levels (Beumer et al.  
52 2006). Recovery can be improved if the radioactivity recovered from cages by washing and,  
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54 30 in the case of the first AFB1 experiment, the radioactivity fixed in the carcasses at the end of  
55 the trial is included in the calculation. Residual activity in carcasses of animals 360 h after  
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58 32 administration of AFB1 was low indicating that a significant amount was eliminated by an  
59 alternative route. Other studies also reported a partial recovery of the total administrated  
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34 radioactivity (Suzuki et al. 1977; Galtier et al. 1979; Coulombe, Sharma 1985; Kumagai et al.

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1998), which could be explained by toxin retention within the kidneys in the case of OTA (Zepnik et al. 2003), or in liver in the case of AFB1 (Wogan et al. 1967) and by exhalation of tritiated water generated by exchange of the labelled  $^3\text{H}$  (Beumer et al. 2006). In our study with tritiated toxins, this latter route of elimination of radioactivity was highly probable.

*In vitro* studies demonstrated the adsorption of different mycotoxins such as AFB1 and OTA to viable cultures of *Saccharomyces cerevisiae* (Devegowda et al. 1994; Meca et al. in press). Preparations containing  $\beta$ -D-glucans portions of the specific strain 1026 exhibited a strong binding capacity *in vitro* for aflatoxins (up to 85%) while being poorer at sequestering OTA (12.4%) (Devegowda et al. 1998). Also, the extent of complexation, i.e. AFB1 > OTA, was not altered by pH values that are close to those found within the digestive tract (Yiannikouris et al. 2006). The *in vitro* binding affinity is in agreement with the *in vivo* results observed here in rats, further supporting the hypothesis that binding is a key property involved in the toxicity-alleviating effects of YCW.

Conversely, no dose effect of YCW was observed in the present study. A possible reason for this result could be the low level of mycotoxin used. The adsorptive capacity of yeast  $\beta$ -D-glucans *in vitro* was shown to be modulated by the amount of mycotoxins added to the medium according to a cooperative phenomenon. Binding of the first mycotoxin molecules induce conformation changes in  $\beta$ -glucans that facilitates access to new sites of fixation improving binding efficiency until saturation of all sites of adsorption (Yiannikouris et al. 2003; Yiannikouris et al. 2004a). In our study, toxin molecules were highly diluted and the minimum level of physical contacts capable to produce cooperative interactions was probably not fully achieved independently of YCW concentration.

The present study was based on measurements of radioactivity alone and provides no information on proportion of parent compounds and their metabolic products recovered in blood, faeces, urine or carcasses. It has to be noted that some of the radioactivity found in faeces in the first hours following administration could have been originated from liver-conjugated metabolites (Ha et al. 1999; Gross-Steinmeyer et al. 2002), particularly for AFB1, that are eliminated via the bile (Bassir, Osiyemi 1967; Kumagai, Aibara 1982). Nonetheless, the decreased absorption and increased excretion observed in our experimental model indicates that the tested preparation may have the potential to protect monogastric animals against exposure to low dietary levels of selected mycotoxins.

*A yeast product alters mycotoxins toxicokinetics***Acknowledgments**

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2 Table 1. Mean toxicokinetic parameters in plasma after a single dose of aflatoxin B1 at two concentrations and ochratoxin A with and without  
 3 co-administration of a yeast cell wall preparation in rats  
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Parameters	Aflatoxin B1 (18.12 µg/kg BW)			Aflatoxin B1 (0.716 µg/kg BW)			Ochratoxin A (3.22 µg/kg BW)		
	Control	YCW-L	YCW-H	Control	YCW-L	YCW-H	Control	YCW-L	YCW-H
<b>C<sub>max</sub> (ng/ml/kg BW)</b>	<b>3.66</b>	<b>1.91</b>	<b>2.26</b>	<b>0.72</b>	<b>0.27</b>	<b>0.35</b>	<b>28.78</b>	<b>20.84</b>	<b>21.47</b>
<b>T<sub>max</sub> (h)</b>	<b>4.0</b>	<b>4.0</b>	<b>4.0</b>	<b>4.0</b>	<b>5.0</b>	<b>4.0</b>	<b>5.0</b>	<b>5.0</b>	<b>5.0</b>
<b>K-elimination (h<sup>-1</sup>)</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>
<b>T<sub>1/2</sub> (h)</b>	<b>53.3</b>	<b>55.5</b>	<b>53.7</b>	<b>63.6</b>	<b>65.4</b>	<b>65.4</b>	<b>63.6</b>	<b>65.4</b>	<b>65.4</b>
<b>AUC 0-72h(ng/ml/kg BW)</b>	<b>129.0</b>	<b>82.4</b>	<b>111.0</b>	<b>32.4</b>	<b>19.8</b>	<b>19.9</b>	<b>1731.3</b>	<b>1260.6</b>	<b>1337.9</b>
<b>AUC 0-∞(ng/ml/kg BW)</b>	<b>131.4</b>	<b>83.9</b>	<b>113.1</b>	<b>32.9</b>	<b>20.1</b>	<b>20.2</b>	<b>1758.5</b>	<b>1279.8</b>	<b>1358.4</b>
<b>ΔAUC 0-∞(%)</b>		<b>36.1</b>	<b>13.9</b>		<b>38.9</b>	<b>38.9</b>		<b>27.2</b>	<b>22.8</b>

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 8  $C_{max}$  = maximum plasma concentration;  $t_{max}$  = time to reach  $C_{max}$ ; K-elimination = elimination constant;  $T_{1/2}$  = terminal elimination half-life; AUC = area under the plasma  
 9 concentration versus time curve from 0 to 72 h, 72 to infinite, and 0 to infinite, respectively. Pharmacokinetic parameters were calculated from a concentration-time curve made  
 10 from samples obtained from three different rats at each sampling point except for  $C_{max}$  and  $t_{max}$  which are the mean of all  $C_{max}$  and  $t_{max}$  values obtained.  
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Table 2. Cumulative excretion of mycotoxins after a single dose of aflatoxin B1 at two concentrations and ochratoxin A with and without co-administration of a yeast cell wall preparation in rats

<i>Experiment<sup>a</sup></i>	Cumulative excretion (% , mean±SD) of radioactivity					
	Urines <sup>b</sup>	Faeces <sup>b</sup>	Total excreted <sup>c</sup>	Cage rinse <sup>c</sup>	Carcass <sup>c</sup>	Average recovery of administered <sup>3</sup> H
<i>Aflatoxin B1 (18.12µg/kg BW)</i>						
Control	8.7±2.9	23.8±5.8	51.7	3.9±1.5	2.9	58.5
YCW-L <sup>d</sup>	4.9±0.8	31.6±3.5	55.0	2.2±0.1	1.8	59.1
YCW-H	4.8±1.3	39.6±27.0	58.1	4.5±0.1	NA	63.7
<i>Aflatoxin B1 (0.716 µg/kg BW)</i>						
Control	6.1±2.0	39.0±3.4	45.1	1.3±0.5	NA	46.4
YCW-L	2.9±1.1 *	59.6±18.2 *	62.5	0.9±0.6	NA	63.5
YCW-H	3.3±1.6 *	61.2±16.9 *	64.5	0.9±0.4	NA	57.8
<i>Ochratoxin A (3.22 µg/kg BW)</i>						
Control	5.6±1.3	16.3±2.6	44.1	5.1±1.2	NA	49.1
YCW-L	6.5±2.3	23.8±4.9 *	49.3	2.4±0.7	NA	51.7
YCW-H	6.7±1.6	28.7±6.4 *	51.8	2.0±0.8	NA	53.8

<sup>a</sup> values are means±SD, n= 5 rats, except for aflatoxin B1 (18.12µg/kg body weight (BW)) where 3 rats were used.

<sup>b</sup> Excreted at 72 h

<sup>c</sup> Recovered at the end of the collection period that was 15, 3, and 10 days for aflatoxin B1 18.12 and 0.716, and ochratoxin A 3.22 µg/kg BW, respectively. NA: not analyzed.

<sup>d</sup> Yeast cell wall preparation at low (YCW-L) and high (YCW-H) concentration. See Materials and Methods for details.

\*  $p < 0.05$  between yeast cell wall treatments and controls for each column within each experiment.

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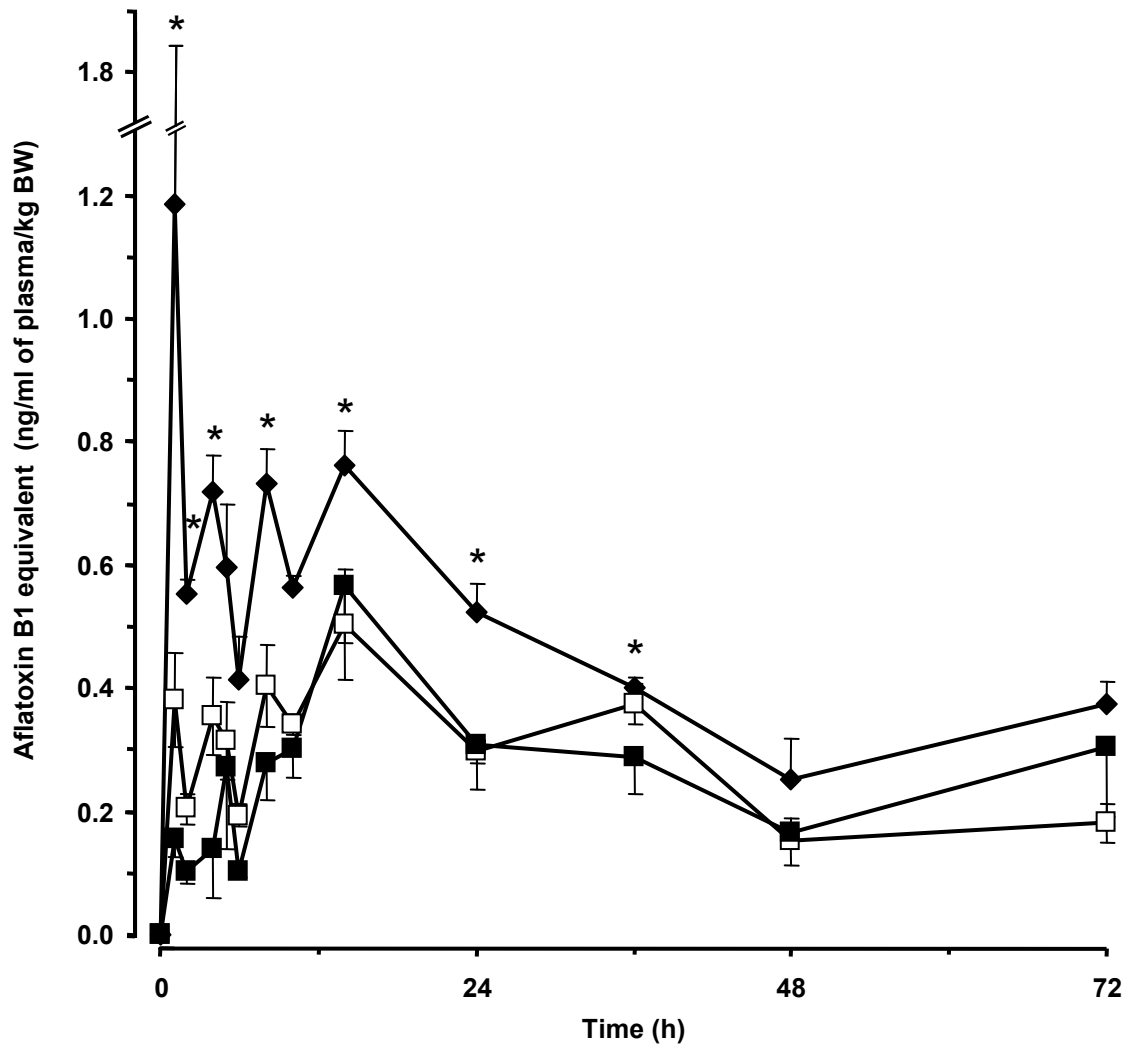


Figure 1. Plasma time-concentration profiles of aflatoxin B1 obtained after a single oral administration of 0.716  $\mu\text{g}/\text{kg}$  body weight with ( $\square$  low and  $\blacksquare$  high) and without ( $\blacklozenge$ ) co-administration of a yeast cell wall preparation in rats. \* indicate  $p < 0.05$  between groups.