Bioavailability of aflatoxin B1 and ochratoxin A, but not fumonisin B1 or deoxynivalenol, is increased in starch-induced low ruminal pH in nonlactating dairy cows

by Dadik Pantaya

Bioavailability of aflatoxin B$_1$ and ochratoxin A, but not fumonisin B$_1$ or deoxynivalenol, is increased in starch-induced low ruminal pH in nonlactating dairy cows

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ABSTRACT

High-production dairy and beef systems require diets rich in starch. This practice may induce ruminal acidosis and also increase exposure to mycotoxins because starches in starch-rich diets are the main vehicles of mycotoxin contamination. The aim of this study was to investigate the effects of low ruminal pH on the bioavailability of 4 major mycotoxins [i.e., aflatoxin B$_1$ (AFB1), ochratoxin A (OTA), deoxynivalenol (DON), and fumonisin B$_1$ (FB1)]. Eight nonlactating dairy cows fitted with rumen cannulas were used in a double crossover experiment. The trial was divided into 4 periods with 2 periods per crossover. Cows were divided into 2 groups receiving a low (15% dry matter basis) and high-starch diet (30.8%) with and without live yeast supplementation (1 × 10^{10} cfu per cow) in the first and second crossover, respectively. At the end of each period, cows received a single dose of mycotoxin-contaminated feed containing 0.05, 0.2, 0.24, and 0.56 mg of AFB1, OTA, DON, and FB1 per kg of feed, respectively. The fecal and urinary excretion of mycotoxins and their metabolites was monitored for up to 48 h postdosing. As expected, ruminal pH decreased in cows fed the high-starch diet. The high-starch diet increased the bioavailability of OTA and AFB1. Urinary excretion of OTA 24 h after mycotoxin administration increased 3-fold in the high-starch diet, correlated with lower fecal excretion. Similarly, a decrease in fecal excretion of AFB1 was accompanied by an increase in urinary excretion of its major metabolite, aflatoxin M$_1$, 48 h after mycotoxin administration. In contrast to AFB1 and OTA, the bioavailability of DON and FB1 remained unchanged. Yeast supplementation had no effect on the excretion balance of these 2 mycotoxins. In conclusion, these results show that high-starch diets increased the bioavailability of OTA and AFB1, most probably through the lowering effect on ruminal pH. This greater bioavailability potentially increases the toxic effects of these mycotoxins.

Key words: mycotoxin, low ruminal pH, dairy cow, live yeast supplementation

INTRODUCTION

High-producing dairy and beef cattle need diets rich in energy and protein to meet their requirements and maximize performance levels. However, the use of large amounts of cereal in the diet may induce SARA, which is often characterized by a decreased ruminal pH (Kleen and Cannizzo, 2012). A starch-rich diet may also increase animal exposure to mycotoxins as starch in these diets are the main vehicles of mycotoxin contamination in feeds. Many reports have indicated the separate negative effects of mycotoxins and SARA in ruminants, but no information is available on any interaction that may exist between a pH-modified ruminal environment as encountered in SARA and the toxicokinetics of mycotoxins. For instance, it has been established that SARA is associated with major changes in the composition of the rumen microbiota (Martin et al., 2006; Fernando et al., 2010; Mao et al., 2013), but it is not known if these changes affect the resistance of ruminants against mycotoxins. Another consequence of SARA is the potential modification of ruminal absorption of mycotoxins. Some authors have reported greater availability of ochratoxin A (**OTA**) in ruminants fed diets rich in starch (Xiao et al., 1991; Blank et al., 2003).

Live yeast used as a direct-fed microbial (**DFM**) exerts a positive effect on the ruminal environment, increasing pH and the numbers of total and cellulolytic

bacteria as well as protozoa (Jouany and Morgavi, 2007; Chaucheyras-Durand et al., 2008). The effects of yeast DFM on ruminal pH and on some ruminal microbes [e.g., protozoa are active degraders of some mycotoxins (Galtier and Alvinerie, 1976)] may indirectly reduce mycotoxin absorption and toxicity. Yeast cell walls also have the capacity to bind to mycotoxins (Shetty and Jespersen, 2006) and thus could reduce gastrointestinal absorption. This property is strain dependent, with reduction of mycotoxin adsorption reported with cell extract preparations (Firmin et al., 2011) but not with a yeast strain tested on OTA in sheep (Blank and Wolffram, 2009).

Mycotoxins such as aflatoxin B_1 (**AFB1**), OTA, deoxynivalenol (**DON**), and fumonisin B_1 (**FB1**) are commonly found in cattle feeds (Rodrigues and Naehrer, 2012) and can cause a variety of toxic responses. Due to their importance on animal health and production, and possible health consequences on humans, the maximum concentration of these mycotoxins in feeds is regulated by the European legislation. The aim of this study was to evaluate the effects of low ruminal pH with and without yeast DFM supplementation on the bioavailability of these 4 mycotoxins.

MATERIALS AND METHODS

Preparation of Mycotoxin-Contaminated Feeds

Aflatoxins and ochratoxins were produced by culture of *Aspergillus flavus* and *Aspergillus ochraceus*, respectively, on wheat as previously described (Boudra et al., 2013). Corn naturally contaminated with fumonisins or DON was obtained locally from an experimental field (Limagrain, Clermont-Ferrand, France). The 4 contaminated batches of wheat and corn were ground to pass through a 1-mm screen and mixed in different proportions to obtain a contaminated cereal mixture containing 3, 12, 15, and 35 mg/kg of AFB1, OTA, DON, and FB1, respectively. Aflatoxins are carcinogenic to humans (group 1) and OTA and FB1 are classified by the World Health Organization International Agency for Research on Cancer as possibly carcinogenic to humans (group 2B). All contaminated batches of wheat and corn were manipulated under a dedicated safety hood for grinding and dose preparation. All personnel wore coats, disposable gloves, protective masks, and goggles.

Animals and Experimental Procedures

The experiment was conducted at the experimental animal facilities of INRA Auvergne-Rhône-Alpes (Saint-Genès Champanelle, France). Animals were cared for in accordance with the guidelines for animal research of the French Ministry of Agriculture and applicable European guidelines and regulations for experimentation with animals (http://www2.vet-lyon.fr/ens/expa/acc_regl.html). The experimental protocol was approved by the Regional Ethics Committee on animal experimentation (CE N° 22612).

Eight nonlactating Holstein cows fitted with a rumen cannula were used. The cows were housed in a tie-stall barn and had free access to water and mineral salt blocks. At the start of the experiment, BW was 650 ± 115 kg. The experiment was a double crossover design with a 2-wk washout period between each crossover to minimize carryover effects. The cows were divided into 2 groups of equivalent BW and age. In the first crossover trial, the cows received a low-starch diet (15.1% DM basis) with or without yeast DFM. In the second crossover, cows received a high-starch diet (30.8% DM basis) with or without yeast DFM. To avoid a possible yeast carryover effect, cows were regrouped for the second crossover with each group having 2 cows that received yeast treatment in the first period and 2 that received yeast in the second period. Each crossover period lasted 3 wk, with a 2-wk adaptation to diet and the last week for measurements. The ingredients and chemical composition of the experimental diets are presented in Table 1. All feed ingredients were tested for presence of mycotoxins. Wheat in the form of pellets was fed at 0700 h, and hay was fed twice daily at 0800 (60%) and 1400 (40%) h. The yeast DFM (*Saccharomyces cerevisiae* CNCM I-1077, Lallemand Animal Nutrition, Blagnac, France) was administered daily before the morning feeding through the ruminal cannula at a dose of 1×10^{10} cfu per cow. Feed offer was limited to 90% of the hay ad libitum intake measured before the start of the trial (DMI is reported in Table 1). At the end of each adaptation period, the cows received a single dose of the contaminated cereal mixture through the ruminal cannula 4 h after the morning feeding, corresponding to the expected nadir in pH. The doses used were 0.05, 0.2, 0.24, and 0.56 mg of AFB1, OTA, DON, and FB1 per kg of feed, respectively. Doses were based on the detection limits of the liquid chromatography-tandem mass spectrometry (**LC-MS/MS**) method used (see below) and all except for AFB1 were below the European Union regulation limits. Each dose of the contaminated cereal mixture (400 g) was divided into 4 equal portions of 100 g and administered through the ruminal cannula in different locations in the rumen to ensure complete ingestion and to facilitate homogenization of mycotoxins with ruminal contents. Total (24 h) and separate collection of feces and urine was performed for 6 d after mycotoxin administration for determination of total-tract digestibility as previously

described (Guyader et al., 2015). To separate urine from feces, cows were fitted with flexible urine cup devices that were attached to the area around the vulva using a hypoallergenic medical adhesive. The cups were connected by a hose to a 30-L plastic carboy. These fecal and urine samples were used for mycotoxin balance measurements. Only the first 2 d are reported because mycotoxins were undetected in longer collection periods. Feed refusals were recorded daily for individual cows for determination of DMI.

Ruminal pH Monitoring, Sample Collection, and Analysis

Ruminal pH was measured continuously throughout the experimental period using commercial boluses (eCow Ltd., Glasgow, UK) as previously described (Guyader et al., 2015). Samples of rumen content, urine, and feces were collected the day before mycotoxin administration and for 2 d thereafter. Total urine in 24 h was collected in a recipient containing 100 mL of 10% H_2SO_4 as a preservative the day before and 2 d after mycotoxin administration. Fractions of 5 mL were transferred into 15-mL polypropylene tubes and stored at −20°C until analysis. Total feces collected over 24 h were homogenized manually, and a representative sample (~100 g) was dried (50°C, 72 h), ground to pass through a 1-mm screen, and stored at −20°C until analysis. Ruminal content samples were taken through the cannula from the reticulum, anterior and posterior areas of the dorsal sac, and posterior of the ventral sac of the rumen and used for determination of fermentation characteristics and mycotoxin analysis. Samples for mycotoxin analysis were collected just before mycotoxin administration (at 0 h) and at 1.5, 3, 6, 12, 24, 48, and 72 h after administration. Two hundred grams of whole ruminal content was added to 100 mL of PBS buffer and homogenized for three 1-min cycles using a Polytron grinding mill (Fischer Scientific, Les Ulis, France). Five grams of homogenized content was transferred to 15-mL polypropylene tubes and immediately frozen to stop microbial activity. Ruminal samples for fermentation metabolites (~100 g) were also collected before and 4 h after the morning feeding just before mycotoxin administration. Ruminal contents were strained through a polyester monofilament fabric (250-µm mesh aperture) and the filtrate was used for analysis. For VFA, 0.8 mL of ruminal filtrate was transferred to a tube containing 0.5 mL of a solution of crotonic acid (4 g/L) and metaphosphoric acid (20 g/L) in 0.5 N HCl and then frozen at −20°C. For lactic acid, 2 mL of ruminal filtrate was transferred to a tube and stored at −20°C. An additional 1 mL of rumen filtrate was collected for ammonia determination, preserved

Table 1. Ingredients and chemical composition of the experimental diets

Item	Low starch	High starch
Ingredient (% of DM)		
Prairie hay	76.2	54.4
Wheat	21.6	43.4
Mineral-vitamin mix[1]	2.2	2.2
Chemical composition (% of DM)		
NDF[2]	53.5	41.1
ADF[2]	31.9	24.5
CP	8.5	10.3
Gross energy (MJ/kg of DM)	17.8	17.9
Starch	15.1	30.8
DMI[3] (kg/cow per d)	10.9	11.0

[1]Minerals (%), P (0.25), Ca (2.0), Mg (0.45), Na (0.35); trace elements (mg/kg): Cu (15); vitamins (IU/kg): vitamin A (6,000), vitamin D_3 (1,250), and vitamin E (10 mg/kg).
[2]Calculated from crude fiber according to INRA equations.
[3]Cows were on restricted intake; they were offered the equivalent of 90% of ad libitum intake measured before the start of the trial on a hay diet. Diet and yeast effects were not significant ($P > 0.05$).

by adding 0.1 mL of 5% ortho-phosphoric acid, and stored at −20°C. For protozoa enumeration, 2 mL of ruminal filtrate was mixed with 2 mL of methyl green formaldehyde solution and stored at room temperature in the dark until counting. Volatile fatty acids were analyzed by gas chromatography as described previously (Morgavi et al., 2003). Lactate was determined using an enzymatic method (Lettat et al., 2010). Ammonia was determined by using the Berthelot reaction (Park et al., 2009).

Mycotoxin Analysis

Sample Extraction and Purification. For urine, 3 mL of distilled water-methanol (10:1, vol/vol) and 3 mL of chloroform-isopropanol (97:3, vol/vol) were added to 15-mL tubes containing 5 mL of urine. The mixture was extracted in a horizontal shaker for 15 min at 40 rpm and centrifuged (3,000 × g, 5 min, 4°C). The top aqueous phase was then removed by aspiration. An aliquot of the organic layer extract (2 mL) was transferred into clean tubes and evaporated to dryness at 45°C under a stream of nitrogen gas. The dried residues were dissolved in 0.2 mL of 0.1% acetic acid in methanol (70:30, vol/vol), vortex mixed for 20 s, and 10 µL of this mixture was then injected into the LC-MS/MS system.

For homogenized ruminal contents (5 g) and feces (3 g), mycotoxins were analyzed according to Lattanzio et al. (2007) with a slight modification. Briefly, the polar mycotoxins FB1, DON, and deepoxy-deoxynivalenol (**DOM1**) were extracted with 25 mL of PBS in

a horizontal shaker for 15 min at 40 rpm, and then centrifuged ($3,000 \times g$, 5 min, 4°C). The decanted supernatant was filtered through a Whatman GF/A glass microfiber filter (Whatman, Lyon, France) and 10 mL was transferred into 15-mL Falcon tubes (extract A). The nonpolar mycotoxins AFB1, OTA, aflatoxin M_1 (**AFM1**), and ochratoxin α (**OTα**) were extracted from the solid residue of the previous extraction with 20 mL of methanol following the method described above for extract A. After centrifugation, 10 mL of supernatant was transferred into a clean polypropylene tube and reduced to ~6 mL at 45°C under a stream of nitrogen gas, then diluted with PBS (20 mL) and filtered through a Whatman GF/A glass filter (extract B).

LC-MS/MS Analysis. The chromatographic system used was an Alliance 2695 module (Waters Corporation, St-Quentin-en-Yvelines, France). Separation was performed at room temperature on a Kinetex C_{18} column (50 × 2 mm, 2.6 µm, Phenomenex, Paris, France) using a solvent system gradient (solvent A = 0.1% acetic acid and solvent B = methanol-0.3% acetic acid). The gradient conditions were as follows: 10% of solvent B was held for 2 min, increased to 15% in 2 min, then to 80% in 4 min, maintained for 7 min, and lowered to the initial percentage in 0.1 min. This 9:1 proportion between solvents was maintained for 5 min to re-equilibrate the column before the next injection. The flow rate was 0.25 mL/min. Electrospray mass spectrometric (**ESI-MS/MS**) analyses were performed on a Quattro Micro triple quadrupole mass spectrometer (Waters Corporation) equipped with an electrospray source operating in positive and negative ion mode. Capillary voltage was set at 3.5 kV, source temperature at 120°C, and desolvation temperature of 350°C. The cone and nebulizer gas flows (both nitrogen) were set at 50 and 500 L/h, respectively. Data were acquired using the multiple reaction monitoring scanning mode. The tune parameters were optimized by separately infusing a 10 µg/mL mobile phase solution of each mycotoxin at a flow rate of 10 µL/min. Two transitions were used; the most intense was used for quantification and the second was used for mycotoxin confirmation. Data were collected in centroid mode at acquisition rate of 0.2 s, with a constant dwell time of 10 ms.

Statistical Analysis

Data on DMI, pH, protozoa, lactic acid, ammonia, VFA, and excretion of mycotoxins were analyzed using the MIXED procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC). Least squares means and standard errors were determined using the LSMEANS statement and differences were determined using Tukey's test post-hoc option with the following mixed linear model:

$$y_{ijk} = \mu + D_i + Y_j + (D \times Y)_{ij} + A(i)_k + \varepsilon_{ijk},$$

where y is the dependent variable and denotes the kth observation at the ith level for factor D and the jth level for factor Y, μ is the overall mean, D is the fixed effect of the diet, Y is the fixed effect of yeast supplementation, D × Y is the diet × yeast interaction, A is the random effect of animal tested within treatment, and ε is the random residual error. The effect of yeast sequence treatment between crossover was not significant and was not further considered. All statements of statistical significance are based on a probability of $P < 0.05$. Trends are discussed at a statistical significance of $P < 0.10$.

RESULTS AND DISCUSSION

None of the cows had any health or behavioral problems throughout the trial. Total apparent DM digestibility was 64.2 and 61.3% for the high- and low-starch diets ($P = 0.03$), respectively, reflecting the differences in the proportions of the highly degradable starch component. Supplementation with yeast increased DM digestibility by 2.8% points in both diets ($P = 0.02$), and no diet × yeast interaction on digestibility was observed. These findings are in agreement with other studies with the same yeast strain (Chaucheyras-Durand et al., 2016).

Ruminal pH and Fermentation Characteristics

Data for ruminal pH (mean, minimum, and time spent below pH 5.6) are shown in Table 2. Cows fed the high-starch diet had lower pH values, and they spent more time under the threshold pH value of 5.6. Supplementation with live yeasts increased mean and minimum pH values ($P = 0.05$) and tended to reduce the time spent under pH 5.6. The modulatory effect of live yeasts on ruminal pH was observed around feeding (just before and up to 1 h after feeding; $P < 0.05$, data not shown) and the effect was numerically observed throughout the day (Figure 1). The effects of diet type and yeast supplementation on ruminal metabolite concentrations were measured before (0 h) and 4 h postfeeding (Supplemental Table S1; http://dx.doi.org/10.3168/jds.2016-11421). Differences were mainly observed before feeding. Cows fed the high-starch diet had higher concentrations of total VFA, with a lower proportion of acetate, whereas yeast supplementation increased the proportion of iso-butyrate and

Table 2. Ruminal pH profile in nonlactating dairy cows fed low- (n = 6) and high-starch (n = 8) diets with (+) and without (−) live yeast supplementation

Variable	Low starch		High starch		SEM	P-value[1]		
	Yeast −	Yeast +	Yeast −	Yeast +		D	Y	D × Y
Mean pH	6.36	6.55	6.21	6.41	0.095	0.052	0.051	0.951
Minimum pH	5.98	6.18	5.21	5.46	0.141	<0.001	0.053	0.799
Time spent pH <5.6 (h/24 h)	0.58	0.44	2.93	2.32	0.868	0.002	0.543	0.676

[1]D = diet effect, Y = yeast effect, D × Y = interaction.

iso-valerate and tended to decrease the proportion of acetate. Lactic acid concentrations were slightly but significantly higher both before and after feeding the high-starch diet. Nevertheless, levels remained within physiological ranges (e.g., ~3 mM 4 h after feeding the high-starch diet). Total numbers of ruminal protozoa were not affected by diet. However, the high-starch diet reduced the concentration of large entodiniomorphs and *Isotrichidae* (Supplemental Table S2; http://dx.doi.org/10.3168/jds.2016-11421).

In this study, a higher proportion of starch in the diet increased the ruminal concentration of total VFA, affected the proportion of acetate and propionate, and decreased ruminal pH as compared with the low-starch diet. These modifications are characteristics of SARA syndrome (Nagaraja and Titgemeyer, 2007) even though the time spent below pH 5.6 was slightly less than the 3-h threshold usually reported in the literature (Gozho et al., 2005; AlZahal et al., 2008). It is noted that pH boluses are located in the reticulum (Mottram et al., 2008), where the pH is 0.24 to 0.70 units higher than the rumen (Sato et al., 2012; Falk et al., 2016). Nevertheless, the difference in ruminal pH between diets was considered adequate for the aims of the study. The choice of diet and restricted intake using nongestating and nonlactating cows was made to induce mild SARA in physiologically stable animals, avoiding difficulties in predicting changes in ruminal pH when acidosis was more severe. The experimental design was also chosen to avoid repeated acidosis as it induces greater individual variations (Dohme et al., 2008). Repeated acidosis also increases fluctuations in ruminal pH and microbial communities that differ between challenges (Dohme et al., 2008; Silberberg et al., 2013). Stability in ruminal characteristics is important when assessing the role of the rumen in absorption and degradation of mycotoxins.

Fate of Mycotoxins After Single Administration

We compared urinary and fecal excretion from cows fed a high and a low starch diet to evaluate whether low ruminal pH affects the bioavailability of mycotoxins in the gastrointestinal tract. In addition, OTA and DON were used as markers of the biodegrading capacity of the ruminal microbiota. These 2 mycotoxins are readily degraded by ruminal microbes into less toxic metabolites that are subsequently found in ruminal contents, feces, and urine (Swanson et al., 1987; Ozpinar et al., 1999). All mycotoxins investigated in this study (AFB1, OTA, DON, and FB1) and their metabolites (AFM1, OTα, and DOM1) were detected in feces and ruminal fluid, whereas only OTA, OTα, and AFM1 were detected in urine. It should be noted that none of these mycotoxins were detected in rumen, urine, or feces before administration of contaminated feeds.

Urinary excretion of AFB1 and OTA was greater for the high-starch diet (Table 3). Fecal excretion of OTA 24 h after mycotoxin administration was more than 3-fold lower in cows fed the starch-rich diet compared with the low-starch diet ($P < 0.002$). This decrease in fecal excretion was correlated with greater urinary excretion of OTA 24 and 48 h after exposure ($P < 0.01$). Similarly, the high-starch diet decreased fecal excretion of AFB1 ($P = 0.03$) and increased that of AFM1, its major metabolite in urine ($P < 0.001$). In contrast to AFB1 and OTA, fecal excretion of DON and FB1 did not decrease when cows were fed the high-starch diet; however, the amount of these mycotoxins increased in feces of cows fed the high-starch diet 48 h after exposure ($P < 0.05$). Although we did not assess the proportion of the administered mycotoxin dose that was available in cows' tissues, results show that lower ruminal pH induced by high-starch diet increased AFB1 and OTA bioavailability. The effects of diet on the bioavailability of OTA was reported by Xiao et al. (1991) who observed a 4-fold increase in OTA absorption in sheep fed high amounts of starch. In a previous study, we tested the disappearance of AFB1, OTA, and FB1 in a temporarily isolated rumen sheep model at acid (pH 5) and neutral pH (pH 7) to evaluate the effects of ruminal acidosis on mycotoxin disappearance (Pantaya et al., 2014). In this isolated rumen model without ruminal contents, and hence reduced load and activity of ruminal microbes, the disappearance rate of OTA and AFB1 was greater ($P < 0.05$) in acidic conditions,

whereas FB1 concentration remained unchanged. In this experiment, we also showed that OTA is absorbed through the ruminal wall because its disappearance from the rumen was confirmed by its Gaussian profile in plasma (Pantaya et al., 2014). In low pH conditions, the increased disappearance rate of OTA and AFB1 suggests the presence of greater proportions of nonionized molecules. The transfer of organic compounds such as mycotoxins through the ruminal epithelium depends on many factors, among which the degree of ionization plays a significant role in the absorption process (Blank et al., 2003). Ionization is mainly influenced by the pH of the medium and only nonionized compounds were absorbed. At pH 5 to 5.5 OTA (acid dissociation constant $pK_a1 = 3.5$ and $pK_a2 = 7$) and AFB1 are partially ionized, whereas FB1 ($pK_a = 3.5$) was completely ionized and therefore not absorbed. This is in agreement with results obtained by Prelusky et al. (1995) who did
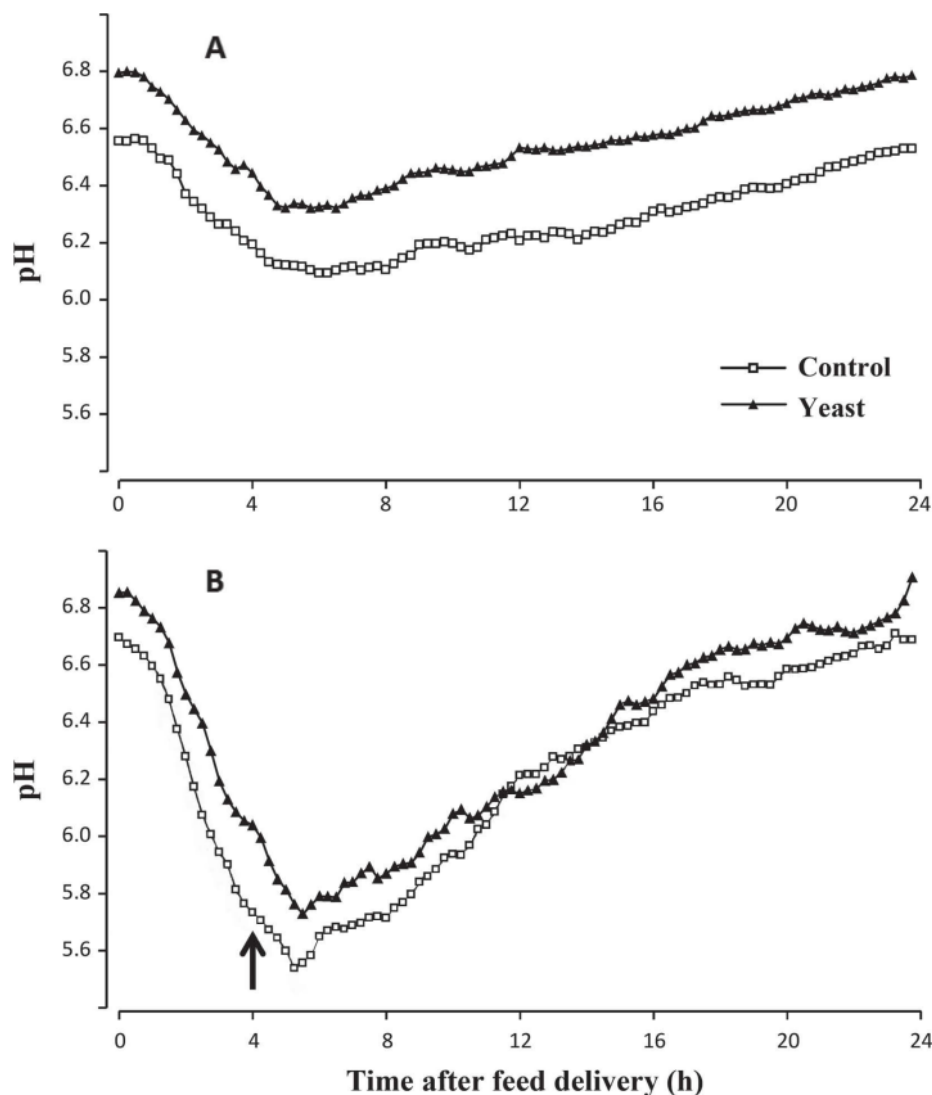

Figure 1. Ruminal pH over a 24-h period in dry cows fed once a day (0 h) low-starch (A) and high-starch (B) diets with (▲) and without (□) live yeast supplementation. Arrow shows time of administration of mycotoxin-contaminated feed.

Table 3. Urinary and fecal excretion of mycotoxins (µg/d; aflatoxin B_1, ochratoxin A, deoxynivalenol, and fumonisin B_1) and their metabolites (aflatoxin M_1, ochratoxin α, and deepoxy-deoxynivalenol 1) in cows (n = 8) fed low- and high-starch diets with (+) and without (−) live yeast supplementation

Mycotoxin[1]	Time[2] (d)	Low starch Yeast −	Low starch Yeast +	High starch Yeast −	High starch Yeast +	SEM	P-value[3] D	P-value[3] Y	P-value[3] D × Y
Urine									
AFM1	1	25.60	8.24	18.35	26.21	10.752	0.589	0.632	0.211
	2	7.70	6.28	16.85	19.36	4.156	0.001	0.847	0.489
OTA	1	0.31	0.19	1.30	1.09	0.338	0.009	0.612	0.895
	2	0.00	0.18	0.66	0.56	0.148	0.001	0.776	0.302
OTα	1	1,170	1,341	1,066	1,400	161.4	0.858	0.060	0.529
	2	825	886	892	928	106.1	0.535	0.585	0.891
Feces									
AFB1	1	12.18	10.65	6.70	9.76	3.019	0.304	0.801	0.455
	2	23.35	23.41	17.98	18.08	2.731	0.031	0.972	0.993
AFM1	1	39.44	32.89	44.10	54.83	10.747	0.114	0.798	0.297
	2	47.38	36.96	49.59	49.60	9.162	0.320	0.484	0.483
OTA	1	11.00	10.98	3.05	4.31	2.390	0.002	0.761	0.752
	2	5.76	9.78	5.41	6.33	2.259	0.296	0.180	0.391
DON	1	471	451	530	531	193.6	0.412	0.912	0.902
	2	109	97	247	425	96.5	0.005	0.282	0.222
DOM1	1	305	289	385	436	100.4	0.197	0.834	0.700
	2	181	86	347	380	111.8	0.038	0.767	0.544
FB1	1	6,050	6,420	4,894	4,145	1,430.5	0.117	0.858	0.599
	2	1,241	1,542	2,617	3,323	510.0	0.002	0.275	0.656

[1]Aflatoxin M_1 (AFM1), ochratoxin A (OTA), ochratoxin α (OTα), aflatoxin B_1 (AFB1), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM1), and fumonisin B_1 (FB1).

[2]Mycotoxins were undetected after 3 d or longer after administration.

[3]D = diet effect, Y = yeast effect, D × Y = interaction.

not find FB1 and its known metabolites in cow plasma after oral administration. Other factors that also have a role are lipophilicity and the size of molecules.

It is recognized that OTA and DON are extensively degraded by ruminal microbes into less toxic OTα and DOM1 (Ringot et al., 2006; Karlovsky, 2011), whereas AFB1 and FB1 remain unchanged in the rumen (Kiessling et al., 1984; Prelusky et al., 1995; Voss et al., 2007). Because ruminal acidosis is associated with significant changes in microbiota composition (Fernando et al., 2010; Silberberg et al., 2013), in this study we also evaluated the ruminal capacity to degrade OTA and DON. No differences were found in OTα and DOM1 between the 2 diets (Table 4). It was clear that high individual variability was probably due to the heterogeneity of the matrix and the difficulty of taking representative samples from whole rumen contents of large animals. We believe that the greater concentration of OTA in high-starch-fed cows at 3 h (P = 0.052) was most probably due to the heterogeneity of the ruminal content as explained above, and not to degradation by ruminal microbes because a concomitant increase did not occur in OTα. The increase in OTA bioavailability in our study was probably due to passive diffusion rather than to a decrease in OTA degradation by ruminal microbiota as reported by Blank et al.

(2003). This is in agreement with results obtained by Xiao et al. (1991) who showed that the disappearance of OTA from the rumen and the corresponding appearance of its metabolite OTα was much faster for hay-fed than for cereal-fed sheep.

No significant differences in the fate of mycotoxins could be observed when live yeast was added to the diets. Live yeast has been reported to be particularly active in the rumen compartment, interacting with the microbiota and thus influencing the ruminal environment (Chaucheyras-Durand et al., 2008). Live yeasts were able to stabilize rumen pH, but as stated above, it is possible that the heterogeneity of mycotoxin concentration in the rumen prevented seeing any possible effect of the yeast DFM on the excretion balance of mycotoxins. This result is in agreement with a previous study (Blank and Wolffram, 2009). Further research is needed to assess the potential action of live yeasts in the fate of mycotoxins in ruminants.

In conclusion, high-starch diets commonly used in intensive dairy and beef production systems to maximize performance levels may increase the bioavailability of mycotoxins by a biochemical mechanism involving a lowered ruminal pH. This study demonstrates that such practices increase the bioavailability of AFB1 and OTA and therefore exacerbate the toxic risk for animals.

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Table 4. Ruminal concentration of mycotoxins (ng/g; aflatoxin B_1, ochratoxin A, deoxynivalenol, and fumonisin B_1) and their metabolites (aflatoxin M_1, ochratoxin α, and deepoxy-deoxynivalenol 1) in rumen juice of cows (n = 8) fed low- and high-starch diets with (+) and without (−) live yeast supplementation

Mycotoxin[1]	Time (h)	Low starch		High starch			P-value[2]		
		Yeast −	Yeast +	Yeast −	Yeast +	SEM	D	Y	D × Y
AFB1	3	0.60	0.70	1.29	0.83	0.339	0.237	0.593	0.412
	6	0.52	0.55	0.54	0.52	0.104	0.953	0.964	0.802
AFM1	3	0.08	0.09	0.06	0.07	0.018	0.229	0.609	0.968
	6	0.06[ab]	0.12[a]	0.04[b]	0.04[b]	0.015	0.001	0.025	0.020
OTA	3	1.05	2.32	7.74	5.44	2.486	0.052	0.830	0.462
	6	0.31	1.90	2.56	1.25	0.786	0.272	0.848	0.054
OTα	3	0.20	0.15	0.16	0.30	0.079	0.470	0.570	0.246
	6	0.18	0.25	0.27	0.30	0.094	0.418	0.530	0.837
DON	3	1.32	2.51	6.72	2.98	1.691	0.085	0.442	0.144
	6	0.17	5.31	1.02	0.00	2.408	0.364	0.402	0.215
DOM1	3	0.54	0.43	0.59	1.03	0.234	0.143	0.415	0.181
	6	0.79	0.72	0.84	1.06	0.269	0.430	0.770	0.543
FB1	3	75.02	59.08	128.51	88.21	25.203	0.093	0.245	0.610
	6	55.11	125.38	55.10	55.91	37.600	0.365	0.355	0.366

[a,b]Different superscripts indicate a significant difference at $P > 0.05$.

[1]Aflatoxin B_1 (AFB1), aflatoxin M_1 (AFM1), ochratoxin A (OTA), ochratoxin α (OTα), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM1), and fumonisin B_1 (FB1).

[2]D = diet effect, Y = yeast effect, D × Y = interaction.

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