Effectiveness of modified yeast cell wall extracts to reduce aflatoxin B1 absorption in dairy ewes

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ABSTRACT

This study investigated the effect of a modified yeast cell wall extract preparation (YCW) on the excretion of aflatoxin B1 (AFB1) and aflatoxin M1 (AFM1) in feces, urine, and milk of dairy ewes fed an aflatoxin-contaminated diet. Sixteen ewes in mid-lactation were assigned to 4 treatment groups: control, AF (60 µg of AFB1/kg of feed), YCW (2 g/kg of feed), and AF+YCW. The trial consisted of a short-term (3-d) exposure period followed by a long-term (21-d) exposure period. At the end of each exposure period, milk, urine, and feces were collected over 72 h. The treatments did not affect feed intake, milk production, milk composition, or body weight. The presence of AFM1 was detected in all matrices, whereas AFB1 was only present in feces. Daily excretion was higher following long-term exposure and reached 26.9 µg of AFB1/d in feces, 37.2 µg of AFM1/d in feces, and 10.7 µg of AFM1/d in urine. Supplementation with YCW was effective in increasing aflatoxin excretion in feces in the long-term exposure (up to 156% increase). The effect was accompanied by a trend of decreasing urinary excretion of AFM1. In contrast, the addition of YCW to the contaminated diet did not affect the transfer of aflatoxins from feed to milk under the present experimental conditions with low-producing ewes. The transfer rates of AFM1 in milk ranged from 0.24 to 0.54%. In conclusion, feed supplementation with YCW reduced the absorption of AFB1 and increased the elimination of AFB1 and AFM1 in ewe feces. Yeast cell wall extract could be used to protect ruminants from chronic exposure to aflatoxins present in feeds.

Key words: aflatoxins, dairy sheep, yeast cell wall extract, excretion

INTRODUCTION

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB1), the most abundant aflatoxin in naturally contaminated foods and feeds, is toxic and carcinogenic to humans and animals. When ingested by ruminants, AFB1 is partly metabolized in the liver to a more polar compound: aflatoxin M1 (AFM1), which is as toxic as the parent toxin and can be excreted in urine, feces, and milk (Allcroft et al., 1967). Both AFB1 and AFM1 are classified as group 2B human carcinogens by the International Agency for Research on Cancer. Aflatoxin contamination occurs frequently in agricultural commodities and poses a major threat to farm animal and human health. In ruminants, toxic effects are associated with liver damage, diminished growth efficiency, diminished milk production and quality, impaired resistance to infectious diseases, and impaired vaccine-induced immunity (Morgavi et al., 2008). Tainted milk and dairy products are the main sources of aflatoxin ingestion by humans in Europe (Prandini et al., 2009).

Several approaches have been investigated to reduce the risk of aflatoxicosis in livestock (Jouany, 2007). Biological, chemical, or physical treatments can minimize toxin production and eliminate contaminants in food and feeds but are not extensively applied in practice due to their cost and limited efficacy (Niderkorn et al., 2007). One approach to reduce the incidence of aflatoxicosis in farm animals is the use of feed additives that restrict the bioavailability of toxins. Mineral adsorbents such as clays or activated carbons have been extensively tested for their potential to complex aflatoxins and impair their gastrointestinal absorption (Kabak et al., 2006). However, these compounds are relatively inefficient toward others mycotoxins and can also impair the absorption of some micronutrients (Huwig et al., 2001).

The use of organic materials such as yeast cell walls is a technological alternative being explored to confront the problem of multi-mycotoxin contamination of feed-stuffs. Among these organic additives, a preparation of chemically modified *Saccharomyces cerevisiae* cell wall

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(YCW), has been shown to adsorb selected major mycotoxins in vitro (Yiannikouris et al., 2004; Shetty and Jespersen, 2006) and to alleviate the effect of dietary mycotoxin exposure in various animal species (Diaz et al., 2004; Chowdhury et al., 2005; Diaz-Llano and Smith, 2006).

Most in vivo experiments performed to date to evaluate the efficacy of YCW adsorbing agents have measured nonspecific parameters such as production performance and the onset of toxicity. Less information is available on how sequestering agents affect toxicokinetics and excretion of AFM1 in lactating dairy ruminants. The objective of the present study was to investigate the effect of a modified YCW extract preparation on the excretion of AFB1 and AFM1 in feces, urine, and milk in mid-lactation dairy ewes fed an aflatoxin-contaminated diet.

MATERIALS AND METHODS

Preparation of Aflatoxin-Contaminated Wheat

The AFB1 used in the study was produced by culture of a toxigenic *Aspergillus flavus* strain kindly provided by Thomas Ostensfeld Larsen from the Technical Center for Microbial Biotechnology, University of Denmark (Kongens Lyngby). Whole grain wheat was adjusted to 34% moisture and placed in a 5-L gas-permeable bag (Full-Gas Microsac, Saco2, Eke, Belgium) and sterilized by autoclaving (121°C, 20 min). Wheat was inoculated with pieces of agar medium from a 1-wk-old culture of *A. flavus*. The bag was incubated at 25°C for 4 wk and then autoclaved (121°C, 20 min) to stop fungal growth; the contents were dried at 80°C for 48 h. The dried grains were then ground to pass a 0.5-mm mesh and analyzed for aflatoxins. Concentrations of AFB1, AFB2, AFG1, and AFG2 in the contaminated wheat were 30 ± 0.1 , 25 ± 1 , 3.5 ± 0.2 , and 6.0 ± 0.2 µg/g (mean $\pm$ SD, $n = 3$), respectively.

Animals and Experimental Design

The experiment was conducted at the animal experimental facilities of the INRA Herbivores Research Unit (Saint-Genès Champanelle, France). Procedures on animals were in accordance with the guidelines for animal research of the French Ministry of Agriculture and all other applicable national and 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 (No. CE4-10).

Sixteen Lacaune dairy ewes in mid lactation at the start of the adaptation period (67.3 ± 3.3 kg of BW, 3.0

Table 1. Ingredients and nutrient composition of the ration fed to the lactating dairy ewes

Item	Amount
Ingredient (% of DM)	
Wheat grain	23.3
Soybean meal	15.3
Prairie hay	56.2
Chemical composition (g/kg)	
DM	87.0
Ash	5.0
NDF	35.7
ADF	16.3
Starch	14.1
Ca	2.5
P	2.7

± 0.9 L of daily milk yield, parity = 3) were used. Ewes were milked at 0730 and 1930 h and fed twice daily (0830 and 1630 h) 1.5 kg of a concentrate mixture plus 2 kg of hay (see Table 1 for ingredients and chemical composition). Feeds were analyzed and the following major mycotoxins were not detectable: AFB1, AFB2, AFG1, AFG2, ochratoxin A, deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin, fusarenon-X, diacetoxyscirpenol, neosolaniol, and zearalenone. Fresh water was available at all times.

After a 14-d adaptation period to the local environment and diet, ewes were divided in 4 treatment groups of similar milk production: control, aflatoxin (AF), YCW (2 g/kg of feed), and AF+YCW groups. The YCW extracts from *Saccharomyces cerevisiae* strain 1026 were provided by Alltech Inc. (purity 90%, batch no. 278 066, Nicholasville, KY). The trial consisted of a 3-d exposure period and 3 d of sample collection that was immediately followed by a 21-d, long-term exposure period and 3 d of sample collection. Each animal was fed a constant dose of AFB1-contaminated wheat at 60 µg/kg of feed. Each daily dose of contaminated wheat was placed in 4 digestible gelatin capsules (1.5 g per capsule), alone or in combination with YCW at a dose of 2 g/kg of feed in a 1:1 ratio (wt/wt), and orally administered with the help of a balling gun before the morning feeding. During both the short- and long-term exposure periods, ewes were placed in metabolic cages for sample collection. Individual daily feed intake and milk production were recorded. Ewes were inspected daily and their weights were recorded weekly. Milk samples were analyzed weekly for butyrate, protein, lactose, and SCC by an external laboratory (CILAL, Theix, France).

Sample Collection

Samples of milk, urine, and feces were taken the day before the last mycotoxin administration and for 3 d

thereafter. Milk samples (100 mL) were collected before (0 h) and 12, 24, 36, 48, and 72 h after the last dose. Milk was preserved by adding 200 μ L of a solution of sodium azide to reach a final concentration of 0.015 g/L and stored at -20°C until analysis. Urine and feces excreted over 24-h periods were collected for 4 d and weighed. Urine was collected in a clean container containing sodium azide (0.5 g/L of urine). A 50-mL sample was centrifuged ($4,000 \times g$, 10 min), aliquoted (1.5 mL) into plastic tubes, and stored at -20°C until analysis. Feces were manually homogenized, and a representative sample of 50 g was transferred into a flask. Fecal samples were dried (50°C , 72 h), ground through a mesh filter (0.5-mm pore size), and stored at -20°C until analysis.

Aflatoxin Analysis

Sample Extraction and Clean-Up. For urine analysis, 2 mL was mixed with 1 mL of methanol and buffered to pH 7.4 with 9 mL of PBS. The mixture was centrifuged ($4,000 \times g$, 10 min) and the whole supernatant was applied to an immunoaffinity clean-up column (IAC, Aflaprep M, R-Biopharm, Lyon, France). The IAC column was then washed with water (10 mL) and air-dried, and the aflatoxins were eluted with methanol (1.5 mL) followed by water (0.5 mL). Fifty microliters of eluate was injected into the HPLC system.

Ground feces (1 g) was suspended in 4 mL of 1% NaCl solution and extracted with 12 mL of chloroform. The mixture was centrifuged ($4,000 \times g$, 10 min), and the organic layer (9 mL) was transferred into a clean tube, where the solvent was evaporated under a gentle stream of nitrogen gas at 50°C . The dried residue was first dissolved in 0.5 mL of methanol, and then 10 mL of PBS at pH 7.4 was added. The solution was centrifuged ($4,000 \times g$, 10 min), and the entire supernatant was loaded onto an IAC column (Aflaprep, R-Biopharm). Clean up procedure was performed as detailed above. Aflatoxins were first eluted with methanol (750 μ L) and evaporated to dryness under a gentle stream of nitrogen gas at 50°C . A second elution was carried out successively with methanol (750 μ L) and water (250 μ L). Fifty microliters of this solution was injected into the HPLC system.

For milk, AFM1 was determined using the official French method (NF EN ISO 1451; Dragacci et al., 2001). Briefly, milk samples were thawed overnight at 4°C , filtered, and loaded onto an IAC column (Aflaprep M, R-Biopharm), which was previously washed with 10 mL of distilled water. Aflatoxin M1 was eluted with 4 mL of acetonitrile. The eluate was evaporated to dryness under a gentle stream of nitrogen gas at 45°C and reconstituted with methanol (125 μ L) and water

(375 μ L). Fifty microliters of this solution was injected into the HPLC system. For each matrix, a validation of the analytical method was performed by analyzing replicates of quality control samples at known concentrations. The following performance criteria: selectivity, linearity, precision and accuracy, as well as recovery and stability of AFB1 and AFM1, were checked and considered satisfactory for all tested matrices (data not shown).

HPLC Procedure. The purified extracts were injected into a HPLC system (Gold 126 Solvent Module, Beckman Coulter Inc., Fullerton, CA) equipped with an automatic injector (AS 3000, Thermo Finnigan, San Jose, CA) and a fluorescence detector (FL 3000, Thermo Finnigan). The detection of aflatoxins was set at 366 nm excitation and 438 nm emission. For each matrix, separation of aflatoxins was performed on the same reversed-phase C_{18} (Nucleodur column, 150 mm $\times$ 4.6 mm) maintained at 40°C and fitted with a C_{18} guard column supplied by Macherey-Nagel (Düren, Germany) by using an isocratic mobile phase: acetonitrile:methanol:water:acetic acid (17.5:17.5:64:1), acetonitrile:water (25:75), and isopropanol-acetonitrile-acetic acid-water (10:10:2:78) set at 1 mL/min flow rate for urine, milk, and feces, respectively. For feces extracts, detection of AFB1 was improved by postcolumn derivatization with pyridinium bromide perbromide at 0.05 mg/mL (Garner et al., 1993).

Toxicokinetics and Statistical Data Analyses

Data on aflatoxin elimination in urine and feces were compared by nonparametric, one-way ANOVA (Mann-Whitney test; Aoudia et al., 2008). Data on BW, DMI, milk production (milk yield, protein, fat concentration, and SCC) and milk AFM1 transfer parameters (concentration, output, and carryover) were analyzed using the MIXED procedure of SAS, with the following mixed linear model:

$$y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + A(i)_k + \varepsilon_{ijk},$$

where y is the dependent variable, μ is the overall mean, T is the fixed effect of the treatment, P is the fixed effect of the sampling period, $T \times P$ is the treatment $\times$ period interaction, A is the random effect of animal tested within treatment, and ε is the random residual. The SCC were divided by 1,000 and converted to the natural logarithm before statistical analysis. All data were analyzed using SAS version 9.1 (SAS Institute Inc., Cary, NC). All statements of statistical significance are based on a probability of $P < 0.05$. Trends are discussed at a statistical significance of $P < 0.10$.

Table 2. Body weight, DMI, and milk production and composition in dairy ewes fed a daily dose of aflatoxin (AF)-contaminated feed, modified yeast cell walls extract (YCW), and their combination throughout the 4-wk experimental period

Production parameter ¹	Treatment				SEM	P-value ²		
	Control	YCW	AF	AF+YCW		T	P	T × P
BW (kg)	67.8	70.3	64.8	62.4	6.73	0.3550	<0.0001	0.9894
DMI (kg)	2.79	2.85	2.79	2.85	0.1708	0.4050	<0.001	0.4166
Milk (L/d)	1.2	1.0	1.3	1.4	0.25	0.2193	<0.0001	1.0000
Fat (g/kg)	82.8	79.3	74.8	80.9	5.40	0.4982	0.0948	0.6558
Protein (g/kg)	63.2	62.0	70.9	62.6	7.07	0.4984	<0.001	0.5994
Lactose (g/kg)	49.3	48.6	46.8	50.1	5.40	0.4982	0.0948	0.6558
SCC ³	6.1	6.0	6.1	5.7	1.25	0.9414	0.2256	0.2218

¹Mean values of each treatment group (n = 4) before and during the short- and long-term exposure periods.

²T = treatment; P = period; T × P = treatment × period interaction.

³Natural logarithmic value of SCC/μL of milk.

RESULTS

Feed Intake, BW, and Milk Parameters

Intake, BW, and milk production were used as generic indicators of the effect of aflatoxin and YCW treatments on ewes. The level of aflatoxins used in this study had no apparent effect on feed intake, BW, or milk production or quality ($P > 0.05$; Table 2). Similarly, no differences were observed for YCW alone or combined with AF compared with controls.

Independently of treatments, daily milk yield decreased by 50% throughout the experiment, whereas DMI and BW decreased by 2.3 and 3.2%, respectively, from beginning to the end of the experimental period ($P < 0.01$). In view of the absence of effects on animal production parameters, the control and YCW treatments were not considered further.

Aflatoxin Excretion After Short-Term Exposure

No aflatoxins were detectable in urine, feces, and milk before administration of contaminated wheat. This finding was expected because ingredients of the

ration were controlled for the absence of aflatoxins and other common mycotoxins.

Transfer of AFM1 into milk 24 h after the last dose decreased numerically with YCW supplementation of the contaminated diet (Table 3). The concentration of AFM1 peaked at the first milking (12 h) after dosing and decreased to trace amounts 72 h after the last dose. Aflatoxin carryover and output were 0.54% and 1.024 μg, respectively, in the AF group; these diminished to 0.40% and 0.760 μg, respectively, in the AF+YCW group.

After the 3-d administration, AFB1 and AFM1 were both recovered in feces in equivalent amounts. In the AF group, they reached their maximum value 24 h after the last administration and decreased to 25% at 72 h postinoculation (Figure 1). Addition of YCW increased by 1-fold the amount of AFB1 eliminated in feces collected before the last administration, but this effect was not observed at other time points. Urinary excretion of AFM1 in the AF group was also maximal 24 h after the last dose and decreased to almost 10% at 72 h postinoculation. Treatment with YCW slightly reduced the elimination of AFM1 in urine (Table 4). In both groups (AF and AF+YCW), individual vari-

Table 3. Aflatoxin M1 (AFM1) transfer in milk of ewes at the last dose of the short-term (3 d) and long-term (21 d) exposure to aflatoxins (AF)-contaminated diet alone or with modified yeast cell walls extracts (YCW)

Item	Short-term exposure		Long-term exposure		SEM	P-value ¹		
	AF	AF+YCW	AF	AF+YCW		T	P	T × P
Milk yield ² (L)	1.2	1.4	0.9	1.0	0.25	0.2554	<0.05	0.537
Output ³ (μg)	1.024	0.760	0.457	0.560	0.2865	0.2622	<0.05	0.0583
Concentration (ng/L)	861	544	520	552	194.3	0.2622	0.0668	0.0583
Carryover ⁴ (%)	0.54	0.40	0.24	0.30	0.150	0.6233	0.0191	0.1839

¹T = treatment; P = period; T × P = treatment × period interaction.

²Mean volume produced 24 h post-dosing.

³Mean AFM1 excreted 24 h post-dosing.

⁴Expressed in terms of AFB1 equivalents according to: molecular weight (MW) of AFB1/MW of AFM1 (312.3/328.3) × μg of AFM1; % calculation based on a daily dose intake of 180 μg of AFB1/d per ewe.

ability among ewes was noticeable, as evidenced by the high standard deviations of the average measures.

Aflatoxin Excretion After Long-Term Exposure

The excretion levels of aflatoxins in both treatment groups after 21 d of administration were higher than those obtained in the same animals after short-term exposure (Figure 1). Recoveries reached 26.9 μg of AFB1 and 37.2 μg of AFM1 in feces and up to 10.7 μg of AFM1/d in urine. In addition, within each group, less variation was observed among individuals.

In contrast to the short-term exposure, after the 21-d exposure a clear positive effect of YCW was observed on fecal excretion patterns (Table 4). The presence of AFB1 and AFM1 was increased in the feces of the AF+YCW group ($P < 0.05$), particularly for AFM1, which increased up to 100% compared with the AF group ($P < 0.05$). This higher excretion of aflatoxins in feces was concomitant with a lower concentration of AFM1 in urine samples compared with concentrations observed in the AF group, although the differences were not significant. In contrast, YCW did not affect the level of AFM1 in milk (Table 3). Concentration profiles of AFM1 in milk and transfer parameters did not differ between AF and AF+YCW groups ($P > 0.05$).

Excretion Balance

For the 3-d exposure, the cumulative amounts of aflatoxins excreted at 72 h posttreatment and expressed as AFB1 equivalents were 111 and 120 μg for the AF and AF+YCW groups, respectively (Table 4). For the 21-d exposure, the quantities increased to 135 and 186 μg for the AF and AF+YCW groups, respectively. This excretion balance provides an indication of the differences between short- and long-term exposures but it should not be confused with toxin recovery because measures were performed after repeated daily doses. Less than one quarter of the recovered aflatoxin was excreted in urine, whereas the amount of AFM1 accumulated in milk did not exceed 1.7% of the total. The excretion balance in the presence of YCW was in agreement with changes observed during the clearance of toxins. The YCW treatment increased fecal excretion of aflatoxins ($P < 0.05$) and tended to reduce AFM1 elimination in urine ($P < 0.10$) during the 21-d exposure.

DISCUSSION

Because of the extent and importance of the mycotoxin problem, the European Union has recently added a new functional group to the technological feed additives category that specifically considers the use of products

“that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action” to improve the safety of feeds (UE/386/2009). Yeast cell wall extracts have been shown to bind aflatoxins and other mycotoxins in vitro (Yiannikouris et al., 2003; Ringot et al., 2007; Sabater-Vilar et al., 2007) and are being studied as feed additives for modulating the toxic effects of mycotoxins on farm animals, including ruminants (Korosteleva et al., 2007; Matur et al., 2010).

Dietary inclusion of YCW has been shown to reduce AFB1 contents in liver of laying hens (Zaghini et al., 2005) and reduce AFM1 secretion in milk in dairy cows (Diaz et al., 2004). We recently showed that YCW increased mycotoxin fecal excretion while reducing plasma concentration in rats (Firmin et al., 2010). In contrast, in other works, the same types of additives did not reduce milk contamination (Stroud, 2006; Waltman, 2008; Kutz et al., 2009) or the presence of AF-albumin adducts in pig plasma (Meissonnier et al., 2009). Because YCW forms complexes with aflatoxin molecules that may reduce toxin absorption, the focus of this work was to test the capacity of a commercial YCW preparation to modify the excretion of AFB1 in feces, urine, and milk in a dairy ewe model of ruminants. It should be noted that the contaminated feed used in this study contained other aflatoxins, notably AFB2. We focused on AFB1 because it is the most common and most toxic food contaminant of the aflatoxin family. The main AFB1 metabolite, AFM1, was also monitored. Other conjugated and oxidative forms of AFB1 may exist but, to our knowledge, they have not been characterized in ruminants and were not monitored. For example, aflatoxicol, another intermediate metabolite of AFB1, can be formed before or after absorption or transformed to other metabolites during transition in vivo (Wu et al., 2009) and was not monitored.

The daily intake levels of 60 μg of AFB1/kg of feed can be compared with the contamination level occasionally found in feeds intended for livestock (Pietri et al., 2004). It should be noted though, that this level was higher than levels legally allowed in dairy animal feeds in Europe (2002/32/CE) and North America (5 and 20 $\mu\text{g}/\text{kg}$ of feed, respectively). The absence of effect on intake, BW, and performance was expected, because the levels used were well below the lower toxic dose. The absence of a negative effect was also in agreement with the literature (Rao and Chopra, 2001; Battacone et al., 2003).

To our knowledge, no recent studies report the excretion of AFB1 and AFM1 in the urine and feces of ruminants. Our results on AFB1 and AFM1 excretion profiles agree with the toxicokinetics described previ-

Figure 1. Daily excretion of (a) aflatoxin B1 (AFB1) in feces, (b) aflatoxin M1 (AFM1) in feces, and (c) AFM1 in urine of ewes measured the day before (1 on x-axis) and for 3 d after (2, 3, and 4) the last dose of aflatoxin-contaminated wheat without (filled bars) or with (open bars) modified yeast cell wall extracts for a short-term (3-d) and long-term (21-d) exposure. Each bar represents the mean $\pm$ SD of 4 ewes. * P < 0.05 between treatments for same day; † P < 0.1 (tendency).

ously by other authors using radiolabeling and thin layer chromatography detection methods. Allcroft et al. (1967) obtained similar excretion patterns in a dairy

cow after a single oral administration of AFB1 (0.5 mg/kg of BW), with most of the excretion occurring within 48 h after administration.

Table 4. Cumulative excretion of aflatoxin B1 (AFB1) and aflatoxin M1 (AFM1; μg , mean $\pm$ SD) in ewes fed an aflatoxin (AF)-contaminated diet alone or with modified yeast cell walls extract (YCW) for a short-term (3 d) and long-term (21 d) exposure

Mycotoxin ¹	Matrix	Short-term exposure		Long-term exposure	
		AF	AF+YCW	AF	AF+YCW
AFM1	Milk	1.9 $\pm$ 0.5	1.5 $\pm$ 0.5	0.9 $\pm$ 0.2	1.1 $\pm$ 0.4
	Urine	17.8 $\pm$ 2.5	15.4 $\pm$ 7.7	26.5 $\pm$ 7.6	16.1 $\pm$ 6.3†
	Feces	57.0 $\pm$ 7.3	49.1 $\pm$ 11.3	57.2 $\pm$ 12.1	98.3 $\pm$ 16.6*
AFB1 Sum ²	Feces	41.7 $\pm$ 9.9	58.1 $\pm$ 7.9*	56.1 $\pm$ 4.1	76.9 $\pm$ 8.7*
	Urine + milk	17.9 $\pm$ 2.4	15.4 $\pm$ 7.1†	25.7 $\pm$ 7.3	15.9 $\pm$ 6.1†
	Feces	96.0 $\pm$ 17.3	104.7 $\pm$ 17.2	109.2 $\pm$ 16.0	170.4 $\pm$ 20.9*
	Total output	111.1 $\pm$ 9.7	120.2 $\pm$ 23.6	134.9 $\pm$ 20.7	186.3 $\pm$ 21.0

¹Excreted in the 24 h before and 72 h after the last dose.²Expressed in terms of AFB1 equivalents according to: molecular weight (MW) of AFB1/MW of AFM1 (312.3/328.3) $\times$ μg of AFM1.† $P < 0.10$ and * $P < 0.05$ between AF and AF+YCW treatments within each row and exposure time.

In our study using a repeated dose design, the cumulative sum of AFB1 and AFM1 accounted for up to 100% of the daily dose at the end of the 3-d clearance period. As previously stated, the repeated dose design is inadequate to calculate the mass balance. Previous investigators reported that after a single dose, only 8 to 9% of the total AFB1 was recovered by urinary and fecal routes in ewes (Nabney et al., 1967), wethers (Armbrecht et al., 1970), or cows (Allcroft et al., 1967).

The addition of YCW to the contaminated diet reduced absorption of AFB1 in both the 3-d and 21-d exposure periods. During the 21-d exposure, the amount of AFM1 excreted into feces increased almost 2-fold in ewes fed the contaminated diet supplemented with the YCW extract compared with the group fed aflatoxins alone (Table 4). This increase in AFM1 in feces was associated with a tendency for lower urinary excretion. Other studies, using organic adsorbing agents and carried out on rats, indicated similar modifications in excretion patterns with an increase of AFM1 excreted in feces (Gratz et al., 2006). Gratz et al. (2006) suggested that AFM1 was formed in rat enterocytes and diffused back into the lumen of the digestive tract where it can bind to sequestering compounds. Aflatoxin M1 in the feces of ewes that was confirmed by liquid chromatography/tandem MS could be produced by the same mechanism because ruminants possess the necessary cytochrome P450 enzyme in the intestine but it also can come from hepatic biotransformation and subsequent enterohepatic circulation (Lynch, 1972; Larsson et al., 1994; Guerre et al., 1996). Although complementary studies will be needed to determine the origin of AFM1, the increase in AFM1 excretion induced by YCW in the long-term exposure in this experiment accounted for an important proportion of the total aflatoxin eliminated from the animal body.

Nevertheless, the increase in aflatoxin excretion in feces did not translate to reduced AFM1 carryover in

milk. The rate of carryover averaged 0.47 and 0.27% for the short- and long-term exposures, respectively. These values were similar to the 0.26% reported by Battacone et al. (2005) on ewes fed a similar dose, but lower than the 0.12% reported by the same authors using a higher dose (Battacone et al., 2003). Carryover has been shown to increase in high-producing cows (Veldman et al., 1992) and the same may occur in ewes. Other reports have also identified milk yield as the main factor contributing to the variability of toxin excretion in lactating animals (Masoero et al., 2007). The ewes used in our study were in mid-lactation at the start of the trial and may be not a good experimental model to test the effect of mycotoxin-reducing additives on milk carryover. We did, however, observe a reduction of AFM1 concentration in ewes fed YCW during the short-term exposure, when carryover was almost double than that recorded during the long-term exposure. Other studies in dairy cows also reported the absence of effect of YCW on milk contamination (Stroud, 2006; Kutz et al., 2009).

The YCW preparation tested by us contained exclusively modified cell walls of *Saccharomyces cerevisiae* strain 1026. This preparation has been demonstrated in vitro to adsorb AFB1 (Devegowda et al., 1998) without dissociating in ruminal fluid (Moschini et al., 2008) and under pH conditions close to those found within the digestive tract (Dawson et al., 2001; Diaz et al., 2002). Our results with ewes also suggest that binding is the mechanism involved in reducing aflatoxin absorption in the gastrointestinal tract of ruminants because no other effect on productions parameters was observed related to the use of YCW alone in the diet.

CONCLUSIONS

A modified yeast cell wall extract changed the excretion balance of aflatoxin-contaminated feed fed to dairy

ewes. The amount of aflatoxin B1 and its derivative aflatoxin M1 eliminated in feces increased, whereas aflatoxin M1 in urine tended to decrease. The observed increase in aflatoxin elimination in feces is a direct indication that aflatoxin absorption was impaired and suggests that dietary supplementation with this yeast cell wall extract could protect ruminants against the toxic effects of aflatoxins in feed.

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