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In vivo release of aflatoxin B1 bound to different sequestering agents in dairy cows

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ABSTRACT: Nine lactating dairy cows, producing 31.08±5.00 kg of milk/cow/day and fed with a Total Mixed Ration (TMR) with an intake of 22.3±0.8 Kg s.s./cow, were used to investigate the resistance of the AFs-SA complex in the rumen and in the gastro-intestinal tract. Two commercial sequestering agents Atox® and Mycosorb® were used. The AFB1 was also mixed to a rumen fluid (R-SA). AFB1 sequestered by Atox®, Mycosorb® and by R-SA were then fed to cows before the morning meal. Milk samples were collected for 6 consecutive milkings and analyzed for AFM1 content. The *in vitro* binding capacity of the two SA were 94.2% for Atox®, 84.3% for Mycosorb® and 71.86% for the R-SA. Both Atox® and Mycosorb® released some of the sequestered AFB1 determining an increase of the AFM1 in milk as soon as in the 1st milking from oral drenching (4.23±7.33; 23.60±8.23 and 46.06±39.84 ppt for Atox®, Mycosorb® and R-SA respectively). The AFM1 (ng/cow) in milk at the 4th milking was lower (66.04, 661.77 and 1613.04; $P<0.05$) in Atox® and Mycosorb® than R-SA, respectively. The percentage release of bound AFB1 were 1.63% for Atox®, 20.27% for Mycosorb® and 50.48% for R-SA.

Key words: Aflatoxins, Dairy cow, Sequestering agents, Carry over.

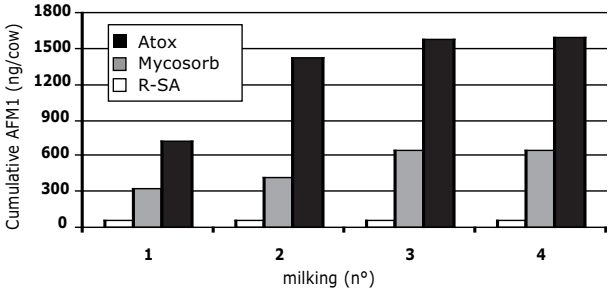
INTRODUCTION – Corn and cotton and their by products are often contaminated by Aflatoxins (AFs), a very toxic and carcinogenic secondary metabolites produced by Aspergillus flavus and A. parasiticus, either in field or in particular storage conditions. Aflatoxin B1 (AFB1) once ingested and absorbed by mammals is rapidly metabolized and excreted into milk as aflatoxin M1 (AFM1). Dietary supplementation with sequestering agents (SA) is the most practical and widely used method to reduce the animal absorption of mycotoxins (Diaz, 2005). Hydrated sodium calcium aluminosilicates (HSCAS) can form an AFs-SA complex impairing the aflatoxin absorption across the intestinal epithelium and therefore reducing negative effects frequently observed in broilers, pig and goats when exposed to mycotoxins contaminated feeds (Phillips *et al.*, 2002). Other products, like commercial Mycosorb® (Alltech Italy, Bologna, Italy), a yeast cell wall extracted glucomannan, are effective toward a large range of mycotoxins (Dawson *et al.*, 2001). HSCAS and yeast cell wall derivates are effective in reducing the AFM1 content in cow milk (Whitlow *et al.*, 2000; Diaz, 2005). Several *in vitro* methods have been proposed to screen SA for their AFs binding capacity, however no information are currently available about the rumen and intestinal resistance of the AFs-SA complex. The objective of this study was to evaluate the milk carry over of the AFB1 previously sequestered either by an Alluminosilicate (Atox®, Grupo Tolsa, Madrid, Spain), yeast cell wall extracted glucomannan (Mycosorb®) or by rumen (R-SA).

MATERIAL AND METHODS - *Preparation of AFs solution* - AFB1 was extracted from a natural contaminated corn meal (82.21±0.01 ppm) using a water/methanol solution (20/80 v/v) in ratio of 1:100 (g:mL), at room temperature and light agitation for 120 minutes. The obtained concentration was 0.821 µg/mL of AFB1. *Preparation of the AFs–SA complex* - Atox® (25 g) and Mycosorb® (75 g) were mixed in 2000 mL water at room temperature, stirred for 60 min and then added 500 mL of the AFs solution. AFs solution (500 mL) was added directly to 2000 mL of rumen fluid (R-SA) collected from a fistulated dairy cow. Then, after 2 hours in light stirring contaminated solu-

tions were centrifuged at 4000 g for 15 minutes. The obtained supernatants were stored at 5°C before AFs analysis. The precipitates were washed with distilled water and then centrifuged until the AFB1 concentration in the supernatant was under 0.05 µg/L. Five washing for Atox®, 15 for Mycosorb® and 16 for the R-AS were required to reach the target concentration. Then, contaminated precipitates were added 900 mL water (37C°), divided in three fractions (300 mL/each) and drenched before morning meal to cows (3 animals/treatment) for a total of 9 animals. *Animals*. The average milk production was 31.08±5.00 kg/day. Animals were fed a Total Mixed Ration (TMR) and had an average dry matter daily intake of 22.3±0.8 Kg. The diet was formulated according to the nutrient requirements of dairy cattle (NRC, 2001) for an average cow weight of 600 kg, 140 days in milk and 33 kg milk yield (3.8% fat and 3.35% protein) and was formed, on a dry matter basis, by: corn silage (25%), alfalfa hay (20%), grass hay (5%) and energy-protein supplement (50%). Cows were milked twice a day (2.30 a.m. and 1.30 p.m.) and individual milk yield was recorded at every milking (Afimilk system, Afikim, Israel). The fistulated cow was fed a TMR based on grass Hay (70%), corn silage (20%) and concentrate (10%) on dry matter basis according to NRC 2001. TMR samples were collected the day before treatments for AFs analysis according to Stroka *et al.* (1999). Individual milk samples were collected at each milking and for consecutive 8 milkings (two before and 6 after treatment) and analyzed by HPLC according to Mortimer *et al.* (1987). The total excretion of AFM1 was calculated. *Statistical Analyses*. Data were analysed by the GLM procedure (SAS) in a complete randomize design.

RESULTS AND CONCLUSIONS - The TMR had a base AFB1 content of 3.45±1.21 ppb contributing to a bulk milk AFM1 content of 15.53±2.54 ppt. The AFB1 washed out from the precipitate were 5.8%, 15.7% and 28.1% respectively for Atox®, Mycosorb® and R-SA. The latter is in agreement with a previous work of Spotti *et al.* (2005) reporting *in vitro* a 45% sequestering capacity of the rumen fluid versus AFB1. Further, the highest efficiency of Atox® observed in this trial confirm our previous data obtained in *in vitro* trial (data not reported) with different AFB1 and SA doses. Inconsistent results were reported on Mycosorb®, either in *in vitro* or *in vivo* trials on different species, suggesting the importance of the experimental condition (pH, buffer characteristics, etc.) on the sequestering capacity of yeast cell wall (Diaz, 2005). In our experiment Mycosorb® sequestred 71.9% of the AFB1, in agreement with previous results from Devegowda *et al.* (1998) and obtained in different experimental conditions. The rumen fluid used in our experiment had a pH of 5.9, not optimal for yeast cell wall derivate activity (Diaz, 2005), however, the high AFs:SA ratio could be the key to get good performance. No works have been published on this matter however, as emphasize by Diaz (2005), a two step *in vitro* trial must be used to evaluate the efficacy of the sequestering agent: the initial sequestration ('weak binding') and desorption ('strong binding').

Figure 1: Cumulative AFM1 excretion in four milkings from drenching



Many authors used chemical solvent extraction, like methanol or chloroform, to evaluate 'strong binding'. The figure 1 clearly state the weak resistance of the R-AFs complex in rumen and in the intestine compared to Atox® or Mycosorb® complexes. An high individual variability was observed between cows, probably due to physiological factors among which there are milk production (kg/day), site of adsorption in the gut, ruminal pH or permeability of mammary gland as previously reported by Veldman *et al.* (1992). The highest AFM1 concentration in milk was detected as soon as the first milking after oral drenching (4.23±7.33 ppt for Atox®; 23.60±8.23 for Mycosorb® and 46.06±39.84 for R-SA). Differences ($P<0.05$) were observed in the AFM1 excreted in milk at the 4th milking (table 1). When considering a carry over rate of 3%, a normal value in high producing dairy cows (Veldman *et al.*, 1992), the AFB1 release from the complex was 1.6, 20.3 and 50.5%, respectively for Atox®, Mycosorb® and R-SA,. Data suggest an interesting efficacy for Atox®, with AFB1 release confined only in the 1st milking (figure 1).
The observed differences on the binding resistance efficacy indicate the need of further application of the technique as tool for screening of sequestering agents. The AFB1 CO observed for the R-SA complex suggest the rumen system is effective only in slowing down the dynamic of adsorption of AFs.

Table 1. AFM1 excreted, carry over (CO, %) and AFB1 released from the AFs-SA complex when feeding AFB1 sequestered into different sequestering agents.

Item		Sequestering Agents			SEM
		Atox®	Mycosorb®	R-SA	
AFM1 excreted at 4th milking	(ng/cow)	66.04[a]	661.77[a]	1613.04[b]	274.69
CO	(%)	0.05[a]	0.61[a]	1.51[b]	0.257
AFB1 released from AFs-SA complex	(%)	1.63[a]	20.27[a]	50.48[b]	8.559

[a,b] *P<0.05.*

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