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10 Mucosal absorption of aflatoxin B1 in lactating dairy cows

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10.1 Abstract

The objective of this experiment was to monitor plasma levels of aflatoxin B1 (AFB1) aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and aflatoxin M1 (AFM1) in lactating dairy cows fed afltaoxin contaminated corn. Seven lactating Holstein cows were given a bolus of a naturally contaminated corn meal assuring an intake of 4.9mg AFB1, 1.01mg AFB2, 10.63mg AFG1 and 0.89mg AFG2. Vitamin A, at 1'000'000 IU, was also added as a biomarker of intestinal absorption. Blood samples were collected at 0, 15, 30, 60, 120, 180, 270 and 360 min after bolus. Plasma was analyzed by HPLC for AFB1, AFB2, AFG1, AFG2 and AFM1 concentrations. Within the considered time points, the peak plasma AFB1 concentration was obtained as soon as 15 minutes from drenching. The plasma AFM1 concentration was considerable as early as the first collection (15 minutes) and peaked at 270 minutes indicating both a rapid absorption of AFB1 through the rumen wall and metabolization into AFM1 in liver. The plasma palmitate level suggests the intestinal contribution to the aflatoxin plasma level after 120 min.

Key words: *Aflatoxins, absorption, blood, cow*

10.2 Introduction

The aflatoxins are secondary metabolites produced primarily by Aspergillus flavus and A. parasiticus. Aflatoxin M1 (AFM1) is the principal oxidized metabolite of AFB1 and it can be readily found in milk and urine of most mammalians after consumption of AFB1 (Wood, 1991). The aflatoxins, as a group (AFB1, AFB2, AFG1, AFG2 and AFM1), are classified as group 1 carcinogens (IARC, 2002). The European Union limits AFB1 allowed in animal feeds, concentrate

and the AFM1 in milk levels greater than 20 ppb, 5 ppb (EC, 2003a) and 0.05 ppb (EC, 2006), respectively. In the US the USDA permitted limit for AFM1 in milk is of 0.5 ppb (Berg, 2003). The AFB1 is promptly absorbed within the gastro intestinal tract of dairy cows (Polan et al., 1974), however, there are no work indicating the site of absorption in the gastro intestinal tract. The objective of this work was to monitor plasma levels of AFB1, AFB2, AFG1, AFG2 and AFM1 in lactating dairy cows following a single bolus of aflatoxins contaminated corn meal to determine sites of absorption.

10.3 Material and Methods

The experiment was carried out using seven lactating Italian Holstein Friesian cows housed at the CERZOO research and experimental center (San Bonico, PC, Italy). The research protocol and animal care were in accordance with the EC council directive guidelines for animals used for experimental and other scientific purpose (EEC, 1986). On the day of experiment animals were given via oral drench and before the morning meal a bolus of naturally contaminated corn meal (97.9±1.41 ppm AFB1, 20.2±0.5 ppm AFB2, 212.6±0.6 ppm AFG1, 17.7±0.3 ppm AFG2). The level of corn contamination assured a total intake of 4.9mg, 1.01mg, 10.63mg and 0.89mg/cow, for AFB1, AFB2, AFG1 and AFG2, respectively. The plasma retinol palmitate was used as a biomarker for the intestinal absorption (Bertoni et al., 2001) following the addition of vitamin A in reason of 1'000'000IU into the drench. Blood samples were taken before morning meal via jugular venipuncture at 0, 30, 60, 120, 180, 270 and 360 min after animal drenching. The blood was collected into Li-Heparinized (17 U of heparin/mL of blood) Vacutainer tubes (Vacutainer systems, Belliver industrial estate, Plymounth, UK). Then, plasma was obtained by centrifugation (3'000 rpm for 15 minutes). The plasma fraction was isolated and stored at -20°C until analyzed for aflatoxins and relative metabolite by High Performance Liquid Chromatography (HPLC) analysis. The aflatoxins extraction was done by the immunoaffinity technique. Aflatoxins were extracted into chloroform (20mL plasma mixed with 90mL of chloroform). Then, the chloroform layer was evaporated to dryness and reconstituted in 1.5mL methanol and 35mL water. The elution were passed through an immunoaffinity column (Aflatoxin Easy-extract, Rhône diagnostics technologies, Glasgow, UK) previously washed with 20 mL of a phosphate-buffered saline solution (pH 7.4). The column was washed with 5 mL water, and slowly eluted with 2.5 mL of methanol. The extract was dried under nitrogen, redissolved in 1 mL acetonitrile:water (25:75) solution and filtered (Millipore Corporation, Bedford, Massachusetts, USA, HV 0.45 μm) before HPLC analysis.

10.4 Results and Conclusions

The plasma AFM1 was detectable (45.1 ppt) as soon as 15 minutes from drenching (table 10-1). Since their low molecular weight (AFB1 = 312.27 and AFM1 = 328.27), the toxins are rapidly adsorbed through membranes by a passive mechanism and the transfer to blood and biological fluids of AFB1 is by passive diffusion of the polar component into the liquid phase and by diffusion or active transport of the non polar component into the lipid phase (Yiannikouris and Jouany, 2002). The AFB1 and AFM1 levels observed just after 15 minutes from drenching indicate both a rapid absorption of AFB1 through the rumen wall and metabolised in the liver into AFM1.

The measured plasma retinol palmitate suggests a probable early AFB1 absorption at rumen level and an AFB1 intestinal contribution to the AFM1 plasma level about 120 minutes after drenching (table 10-1 and figure 10-1). The AFB1:AFM1 ratio (1:3.4) was lowest at 270 from the beginning of treatment.

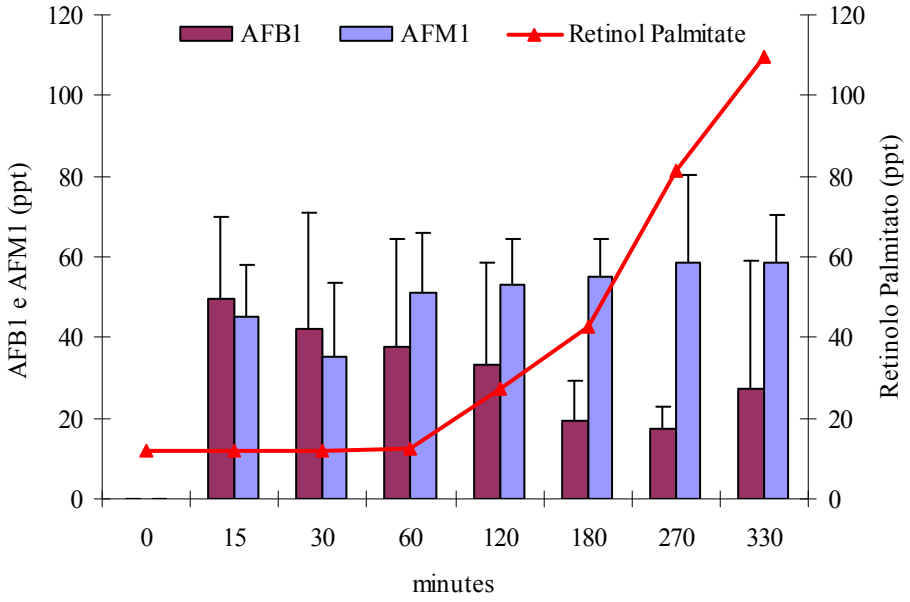
The data reveal important information for a better understanding of place and dynamics of aflatoxin absorption in dairy cattle that could be useful to minimize the AFM1 residues in products intended for human consumption.

Table 10-1. AFB1, AFB2, AFG1, AFG2, AFM1 and retinol palmitate plasma levels following treatment bolus drenching

Item	Time, minutes							
	0	15	30	60	120	180	270	360
AFB1, ppt	n.d.	49.5±21	42.2±29	37.8±27	33.0±26	19.2±10	17.4±5	27.2±32
AFB2, ppt	n.d.	9.0±7	8.0±8	6.8±5	9.2±8	5.5±3	4.4±3	7.2±8
AFG1, ppt	n.d.	24.2±17	20.2±14	24.1±21	48.0±82	14.6±10	12.5±7	43.8±84
AFG2, ppt	n.d.	3.6±3	3.2±3	3.6±2	8.5±10	2.8±2	2.7±2	7.8±10
AFM1, ppt	n.d.	45.1±13	35.3±19	51.0±15	53.2±11	55.1±9	58.7±22	58.7±12
Retinol palmitate, ppb	12.3±2	-	-	12.4±2	23.8±6	43.9±7	86.8±49	109.3±6

n.d. not detectable

Figure 10-1. AFB1 and AFM1 (ppt) and Retinol Palmitate (ppb) pattern after treatment bolus drenching



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11 Aflatoxin B1 absorption in the gastro-intestinal tract and in the vaginal mucosa in the lactating dairy cows

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11.1 Abstract

The objective of the experiment was to monitor plasma levels of aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) and M1 (AFM1) in lactating dairy cows fed a single oral bolus with aflatoxin natural contaminated corn meal (Trial 1). The possible aflatoxins (AFs) absorption through mucous membranes was also investigated using the vaginal mucosa (Trial 2). In trial 1, seven lactating Holstein dairy cows were given a single oral bolus of a naturally contaminated corn meal assuring an intake of 4.89 mg AFB1, 1.01 mg AFB2, 10.63 mg AFG1 and 0.89 mg AFG2. Blood samples were collected at 0 and 5, 10, 15, 20, 25, 30 minutes after treatment. In trial 2 a similar aflatoxin dosage of trial 1 was provided through vaginal implant to eight lactating Holstein dairy cows.. Blood samples were collected at 0 and 15, 30, 60, 180, 360 minutes after treatment. Individual milk samples of six milkings, one before and five after treatment, were also collected. Plasma and milk samples were analyzed by HPLC for AFB1, AFB2, AFG1, AFG2 and AFM1 contents. In trial 1 AFB1 in plasma peaked (33.6 ng/L) as soon as 20 minutes after treatment. The plasma AFM1 was already detectable at 5 minutes (10.4 ng/L) and peaked at 25 minutes (136.3 ng/L). In trial 2 only AFB1 and AFM1 were detectable in plasma, starting from the first sampling time (15 minutes), with values of 10.7 and 0.5 ng/L, respectively. The AFB1 peaked at 30 minutes (23.9 ng/L). The AFB1 excreted in milk as AFM1 had the highest concentration (203.0 ng/L) in the first milking after treatment and decreased close to the starting values after 36 hours from treatment.

The prompt appearance of studied aflatoxins, and their metabolites, in plasma suggests absorption might also take place in mouth or oesophageal mucous membranes, before the rumen compartment.

Results support the hypothesis that the cytochrome P450 oxidative system, which is present in these tissues and in leukocytes, could be involved in the conversion of the AFB1 in AFM1. The

absorption of AFB1 through the vaginal mucosa confirms the passive diffusion as a probable mechanism for AFB1 absorption.

Key Words: *Aflatoxin B1, Aflatoxin M1, Dairy cows, Absorption, Blood, Milk*.

11.2 Introduction

Aflatoxins (AFs) are secondary metabolites produced principally by Aspergillus flavus and A. parasiticus either in pre or post-harvest conditions (Samapundo et al., 2007). These fungi can colonize and produce AFs on many food and feed products such as corn, cotton and peanut. The presence of AFs on feedstuffs can be severe and cause significant economic losses and potentially raise public health concerns (Norton, 1999). A. flavus and A. parasiticus are known to produce mainly four types of AFs: aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) (Scheidegger and Payne, 2003).

The AFB1 is considered one of the most potent known natural hepatic-carcinogen for mammals (Creppy, 2002). When adsorbed by lactating animals, the AFB1 is hydroxylated and its main metabolite, the aflatoxin M1 (AFM1), is excreted in the urine, feces and milk (Yiannikouris and Jouany, 2002).

The liver is the main site of AFB1 bio-transformation with the mitochondrial cytochrome P450 oxidative system (CYP) converting the AFB1 into AFM1 and other metabolites (Sudakin, 2003). The AFM1 preserves the same acute toxicity of AFB1 in rats (Pong and Wogan, 1971), with lower carcinogenic potential (Wogan and Paglialunga, 1974). Thus, the metabolic pathway leading to AFM1 formation can be considered a detoxification process (Yannikouris and Jouany, 2002).

The IARC (2002) classified AFB1, AFB2, AFG1, AFG2 and AFM1 as carcinogenic to humans (Group 1). The European Union allowed maximum limits for AFB1 concentration in feed materials, complete feedingstuffs and AFM1 in milk are set at 20 μg/kg, 5 μg/kg (2003/100/EC, 2003) and 0.05 μg/kg (2006/1881/EC, 2006), respectively.

The animal exposure to AFB1 occurs mainly with the ingestion of contaminated feeds (Sudakin, 2003), however skin (Rastogi et al., 2006) or inhalation (Jakab et al., 1994) exposures might also contribute. When absorbed, AFs presence in blood is prompt (Trucksses et al., 1983; Coulombe and Sharma, 1985) and can reach organs and peripheral tissues likely through a passive mechanism (Yannikouris and Jouany, 2002). The parent toxins (AFB1, AFB2, AFG1, AFG2) or the AFM1 have been detected in plasma of cows as soon as 15 minutes from oral administration of AFs naturally contaminated corn meal (Moschini et al., 2007).

The objective of this work was to monitor the early plasma levels of AFs in lactating dairy cows following a single oral bolus of AFs contaminated corn meal. The possible AFs absorption through mucous membranes was also investigated using the vaginal mucosa.

11.3 Materials and methods

11.3.1 Animals and samplings

Two experiments were carried out on lactating Italian Holstein Friesian dairy cows (seven cows in the first trial and eight cows in the second trial). Cows were housed at the CERZOO research and experimental center (San Bonico, Piacenza, Italy). The research protocol and animal care were in accordance with the European Community council directive guidelines for animals used for experimental and other scientific purpose (EEC, 1986).

Cows were housed in a free stall barn and had free access to water. 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 (DIM) and a 35 kg milk yield (3.80% fat and 3.35% protein). The bulk of the diet on a dry matter basis was: corn silage (31.2%), dehydrated alfalfa hay (16.7%), grass hay (4.1%) and energy-protein supplement (48%). The diet was fed once a day (0900 h) and ad libitum (5% expected orts) as a total mixed ration (TMR).

Cows were milked twice a day (0230 h and 1330 h) and the individual milk yield was recorded at every milking (Afimilk system, Afimilk, Israel).

11.3.2 Trial 1: Aflatoxin as oral drench

On day of experiment seven cows averaging 185±43 DIM and 28.75±4.37 kg milk yield (mean ± sd) were given by oral drench and before the morning meal a bolus of AFs naturally contaminated corn meal for a total intake of 4.89 mg, 1.01 mg, 10.63 mg and 0.89 mg, respectively for AFB1, AFB2, AFG1 and AFG2. Cows were blood sampled by jugular venipuncture at 0, 5, 15, 20, 25, and 30 minutes from treatment. The blood was collected into Li-Heparinized (17 U of heparin/mL of blood) Vacutainer tubes (Vacutainer systems, Belliver Industrial Estate, Plymounth, UK). Then, plasma was obtained by centrifugation (3’000 g for 15 minutes) and stored at -20°C until AFs analysis by High Performance Liquid Chromatography (HPLC)

11.3.3 Trial 2: AFs administration in vagina

Eight cows averaging 192±48 DIM and 27.78±4.13 kg milk yield (mean ± sd) were used in the experiment. AFs were extracted from 50 g of naturally contaminated corn meal with a methanol:water solution (80:20 v/v). Then, the methanol:water extract was dried under nitrogen, redissolved into 20 mL of water and adsorbed to a cotton wad. The cotton wad containing 4.89 mg AFB1, 1.01 mg AFB2, 10.63 mg AFG1 and 0.89 mg AFG2 and was implanted directly in the vagina of the cows before the morning meal.

TMR samples were collected the day before treatment for AFs content determination. Individual milk samples were collected from four randomly selected cows at each milking and for six consecutive milking (the first sample before treatment). Then, samples were frozen at -4°C before HPLC analysis.

Blood samples were taken before morning meal by jugular venipuncture at 0, 15, 30, 60, 180 and 360 minutes after treatment and collected into Li-Heparinized (17 U of heparin/mL of blood) Vacutainer tubes (Vacutainer systems, Belliver Industrial Estate, Plymounth, UK). Then, plasma was obtained and stored as in trial 1.

11.3.4 Sample Analysis

Extraction of the AFs from feeds was done by the immunoaffinity technique according to Arranz et al. (2006). Briefly, ten grams of dried feed were put in a methanol:water solution (80:20 v/v), shaken at 150 rpm for 45 minutes (Universal table Shaker 709) and filtered with Schleicher & Schuell 595 ½ filter paper (Dassel, Germany). Then, five mL were eluted with 45 mL of bi-distilled water through an immunoaffinity column (Aflatoxin Easy-extract, Rhône diagnostics technologies, Glasgow, UK) previously washed with 20 mL of a phosphate-buffered saline solution (pH 7.4). The column was washed with five mL water and slowly eluted with 2.5 mL of methanol. The extract was dried under nitrogen, redissolved in one mL acetonitrile:water (25:75) solution and filtered (Millipore Corporation, Bedford, Massachusetts, USA, HV 0.45 μm) before HPLC analysis.

Extraction of the AFs from milk was done by the immunoaffinity technique according to Mortimer et al. (1987). Briefly, 50 mL of defatted milk (centrifuged at 7'000 rpm for 10 minutes at 4°C) were filtered with Schleicher & Schuell 595 ½ filter paper (Dassel, Germany). Then, 20 mL were passed through an immunoaffinity column (Aflatoxin Easy-extract, Rhône diagnostics technologies, Glasgow, UK) previously washed with 20 mL of a phosphate-buffered saline solution (pH 7.4). The column was washed with five mL water, and slowly eluted with 2.5 mL of methanol.

The extract was dried under nitrogen, redissolved in one mL acetonitrile:water (25:75) solution and filtered (Millipore Corporation, Bedford, Massachusetts, USA, HV 0.45 μm) before HPLC analysis.

Extraction of the AFs from plasma samples was done by the immunoaffinity technique. Obtained sample volume was recovered and plasma was put in a separatory funnel with 35 ml of chloroform. Then chloroform phase was separated and evaporated with Rotavapor (Büchi Labortechnik, Postfach, Switzerland). After the evaporation, AFs extracted were dissolved with one ml of methanol and 35 ml of water and the solution was passed through an immunoaffinity column (Aflatoxin Easy-extract) previously washed with 10 mL of a phosphate-buffered saline solution (PBS/2%, pH 7.4). The column was washed with five mL water, and slowly eluted with 2.5 mL of acetronitile. The column extract was dried under nitrogen, redissolved in one mL acetonitrile:water (25:75) solution and filtered before HPLC analysis.

11.3.5 Chromatography

The HPLC analysis was performed by a Perkin Elmer LC (Perkin Elmer, Norwalk, CT, USA) equipped with a LC-200 pump and a Jasco FP-1520 fluorescence detector (Jasco, Tokyo, Japan). The system and data acquisition were controlled by Jasco Borwin Chromatography PC software.

The AFs in feed and in plasma was separated with a reverse-phase C18 Superspher column (4 μm particle size, 125 x 4mm i.d.; Merck, Darmstadt, Germany) at room temperature and isocratic conditions, with a mobile phase of water and acetonitrile:methanol solution (17:29 v/v) with a 64:36 (v/v) ratio. The flow rate was 1mL/min. Then, the AFB1 was detected by fluorescence, after postcolumn derivatization (Jasco 2080 Plus HPLC pump) with pyridinium hydrobromide perbromide (PBPB) at flow 0.1 mL/min. The fluorescence detector was set at 365nm excitation and 440nm emission wavelengths. The standard stock solution was checked for AFB1 concentration according to A.O.A.C. method 970.44 (AOAC, 1995) and stored at -20°C when not in use.

The AFM1 was separated with a reverse-phase C18 LiChospher 100 column (Merck, Darmstadt, Germany, 5 μm particle size, 125 x 4 mm I.D.) at room temperature, with a water and acetonitrile (75:25 v/v) mobile phase made and the flow rate set at 1 mL/min. The fluorescence detector was set at 365nm excitation and 440nm emission wavelengths. The standard stock solution was checked for AFM1 concentration according to A.O.A.C. method 970.44 (AOAC, 1995) and stored at -20°C when not in use.

11.3.6 Statistical Analysis

Statistical evaluation of AFB1, AFB2, AFG1, AFG2 and AFM1 plasma and milk levels were carried out by paired t test. Differences between values at each sampling time after treatment versus the value at time zero were computed for considered parameters. By using the proc means of SAS (V9.1, Statistical Analysis Systems Institute Inc., Cary, NC) a mean and standard error of the obtained differences were computed for each parameters and the probability that the absolute value of the mean difference was greater than zero by chance alone was estimated.

11.4 Results and discussion

11.4.1 Trial 1: Aflatoxin as oral drench

This work follows a previous study on dairy cows (Moschini et al., 2007) in which the AFB1 and AFM1 were detectable in plasma as soon as 15 minutes after the ingestion of an AFs contaminated corn meal bolus.

In the current trial the TMR had a AFB1, AFB2, AFG1 and AFG1 contents of 1.7±0.4, 0.3±0.1, 0.5±0.2 and 0.1±0.1 μg/kg respectively, resulting in a non detectable level of AFs in plasma of lactating dairy cows (table 11-1).

The AFB1 ($P < 0.05$) was found in plasma as soon as five minutes after AFs ingestion (table 11-1). The result suggests a rapid absorption of AFB1 through the gastro-intestinal tract of cows and a quick oxidation of the toxin to AFM1, with a significant increase in plasma at 10 minutes compared to plasma samples collected before treatment ($P < 0.01$). As previously reported (Moschini et al., 2007), an early AFB1 absorption before the rumen wall could be involved before the intestinal contribution for AFB1 absorption, around 120 to 180 minutes after AFB1 ingestion.

For the studied sampling schedule of 0, 5, 15, 20, 25 and 30 the AFB1 plasma level was maximum (33.6 ng/L) at 20 minutes after exposure to AFB1, whereas the AFM1 peaked at 25 minutes from AFB1 exposure (136.3 ng/L). The AFB1 results agreed with our previous data in which the AFB1 plasma level peaked after 15 minutes from treatment (Moschini et al., 2007).

The AFM1 plasma levels reported by Moschini et al. (2007) were 45.3 ng/L at 15 minutes and 35.3 ng/L at 30 minutes from treatment. As in table 11-1, AFM1 plasma concentrations in the present work were 68.0 ng/L at 15 minutes and 135.3 ng/L at 30 minutes. Considering the standard deviations, the AFM1 values at 15 minutes could be considered similar, while a difference could be

addressed for the 30 minutes samples, with values four times higher in the present work and with, the same aflatoxin dosage being given to animals (4.89 mg/cows of AFB1).

However, differences in plasma AFs levels could result from high individual variability among animals, probably related either to differences in plasma volumes or to a different passage of the toxin through the membranes (Van Egmond, 1989; Veldman et al., 1992; Masoero et al., 2007) .

AFB2 and AFG1 were also detectable in plasma five minutes after the oral drench, with the maximum level at 30 minutes. Even in presence of higher AFG1 intake compared to AFB1 (10.63 vs. 4.89 mg/cow), in plasma the AFG1 content was lower than AFB1. The plasma level might be related to differences on absorption dynamics of the parent molecules. Very low concentrations and close to the detectable limit were observed for the AFG2 (table 11-1).

The AFM1/AFB1 plasma ratio moved from 0.99 at five minutes to 3.05 at 15 minutes and to 4.89 at 30 minutes from drenching. The pattern indicates an AFB1 conversion rate to AFM1 which overcomes the body capacity of getting rid of the AFM1, either through faeces, urine and milk (Yiannikouris and Jouany, 2002), and could lead to an accumulation of the metabolite within the blood pool.

The observed prompt absorption of the considered AFs and their appearance in blood, also as metabolite, are in agreement with previous works (Polan et al., 1974; Trucksess et al., 1987; Moschini et al., 2007). Authors suggested a rumen contribution to the AFs absorption before the intestinal tract.

Other ways of AFs passage to the blood compartment could be addressed too. In particular Coulombe and Sharma (1985), working with rats exposed to a single intra-tracheal or oral [H3]AFB1 doses, measured a peak AFB1 plasma concentration after one and three hours from treatment, respectively. Authors suggested that the absorption of non polar and lipid-soluble compounds, like AFs, is a rapid process either through the pulmonary tissues or in the gastro-intestinal tract.

Current results on plasma AFs and their metabolite presence as soon as five minutes after treatment suggest a rapid absorption even before the rumen compartment, through the mouth or oesophageal mucous membranes.

Once absorbed, the AFB1 is converted to AFM1 and other metabolites through processes mainly microsomal CYP mediated (Coulombe, 1993; Yannikouris and Jouany, 2002; Sudakin, 2003), and in particular in CYP3A4 and CYP2A6 forms (Gallhager et al., 1996; Pelkonen et al., 2000). These oxidative systems can be found in different tissues beyond the liver, like small intestine, pancreas, brain, lung, adrenal gland, kidney, bone marrow, mast cells, skin, ovary, testis and leukocytes (Krishna and Klotz, 1994; Chang and Kam, 1999; Lind et al., 2003). Thus, the

oxidation of the AFB1 seems related to either hepatic or extra-hepatic CYP (Coulombe, 1993). It has been reported a high oxidation activity of AFB1 within olfactory and respiratory tissues of cattle, sheep, swine and rat (Coulombe and Sharma, 1985; Tjälve et al., 1992; Larsson et al., 1994; Larsson and Tjälve, 1996). Thus, extra-hepatic conversion of the adsorbed AFB1 might justify the prompt appearance of AFM1 in plasma (table 11-1).

11.4.2 Trial 2: Aflatoxin administration in vagina

The TMR had an AFB1, AFB2, AFG1 and AFG2 contents of 2.3±0.5, 0.4±0.1, 0.9±0.3 and 0.2±0.1 µg/kg respectively, resulting in non detectable levels of AFs in plasma and in a a bulk milk AFM1 content of 2.3±2.0 ng/L of milk from exposed dairy cows.

The AFB1 and AFM1 plasma concentrations following the aflatoxin vaginal implant is reported in table 11-2. The AFB2, AFG1 and AFG2 were not detectable in collected plasma samples.

Both AFB1 and AFM1 were detected in plasma as soon as 15 minutes after treatment. For the considered sampling schedule, the maximum level for AFB1 (23.9 ng/L) was measured at 30 minutes from treatment, whereas no trend seemed detectable for AFM1, with similar concentrations in samples collected between 30 and 360 minutes from treatment (table 11-2).

The low molecular weight (312.27 formula weight) and the lipophilic characteristic of AFB1 could allow a mucosal passive diffusion through tissues as previously reported in different species (Kumagai, 1989; Coulombe, 1993; Hiesh and Wong, 1994; Yiannikouris and Jouany, 2002). Indeed there is a report of a faster pulmonary tissues AFB1 absorption in male rats compared to the gastro-intestinal tract toxin absorption (Coulombe and Sharma, 1985). These observations confirmed that the absorption and biotransformation rate of AFB1 could be affected by the administration route (Hsieh and Wong, 1994).

The prompt and continuous passage of the toxin through the vaginal mucosa observed in our trial might corroborate a passive diffusion process as a mechanism regulating the absorption of AFB1. This in agreement with Kumagai (1989), injecting [H3]AFB1 directly in stomach and in various sites of the small intestine, who found a rate of AFB1 uptake from gut to mesenteric venous blood nearly proportional to the AFB1 concentration.

The highest AFM1 concentration (203.0 ng/L) was measured in milk at eight hours after the treatment (figure 11-1). Then, the pattern of the AFM1 concentration in milk was downward through consecutive milk samples (80% lower in the second sample) up to a similar pre-treatment value at 32 hours from treatment. The AFM1 excretion pattern in milk were comparable to previous

reported data as consequence of a single AFB1 ingested dose (Battaccone et al., 2003) with maximum concentration in the first milking after dosing.

No AFM1 due to AFB1 administration was detected in the fifth milking after treatment.

The AFB1 in milk was found only in samples eight hours after treatment and at very low concentration (4.3 ng/L). The AFB1 was usually excreted in milk in buffalo cows fed an aflatoxin contaminated diet (Pietri et al., 2003), whereas in dairy cows the parent molecule was found in milk only following an AFB1 ingestion higher than 300 mg (Truckess et al., 1983). Our unpublished data confirm that lactating dairy cows ingesting 5, 15 and 50 mg of AFB1 excreted 107.2, 27.1 and 7.8 μg of AFB1 in milk, respectively.

11.5 Conclusions

AFs were quickly absorbed through the gastro-intestinal tract of cows. An early absorption might also take place in mouth or oesophageal mucous membranes, before the rumen compartment. Results support the hypothesis that the CYP oxidative system which is present in these tissues and in leukocytes could be involved in the conversion of the AFB1 in AFM1.

The absorption of AFB1 through the vaginal mucosa confirms a passive diffusion as a probable mechanism for AFB1 absorption.

Table 11-1. Plasma aflatoxins concentration (means ± standard deviation; ng/L) before and after an aflatoxin contaminated oral bolus[1] (n=7)

	Minutes from the oral bolus						
Aflatoxins	0	5	10	15	20	25	30
Aflatoxin B1 (AFB1)	n.d.[2]	10.5*±9.4	21.1*±20.2	22.3***±16.5	33.6***±22.8	25.7***±11.3	27.7***±14.7
Aflatoxin B2 (AFB2)	n.d.[2]	1.3*±1.6	3.2*±3.1	4.5*±4.4	3.7*±4.2	5.9±7.3	7.5*±8.9
Aflatoxin G1 (AFG1)	n.d.[2]	4.6*±5.1	12.0*±14.1	16.3±22.7	15.7±20.4	24.3±36.3	29.0±46.0
Aflatoxin G2 (AFG2)	n.d.[2]	0.5±0.7	0.7±0.8	0.3±0.6	0.7*±0.8	0.9*±1.0	0.6±0.9
Aflatoxin M1 (AFM1)	n.d.[2]	10.4±20.5	55.7**±49.8	68.0***±53.9	109.1***±54.0	136.3***±76.6	135.3***±81.7

[1]Aflatoxins presence into the oral bolus: 4.9 mg AFB1, 1.01 mg AFB2, 10.63 mg AFG1 and 0.89 mg AFG2.

[2]n.d.= not detectable.

Differences with initial value (0 minute) being greater than zero. * $P < 0.05$; ** $P < 0.01$ *** $P < 0.001$.

Table 11-2. Plasma aflatoxins concentration (means ± standard deviation; ng/L) before and after the aflatoxin contaminated vagina implant[1] (n=8)

	Minutes from vaginal implant					
Aflatoxins	0	15	30	60	180	360
Aflatoxin B1 (AFB1)	n.d.[2]	10.7***±8.4	23.9*±27.2	11.8***±10.2	7.8*±7.0	4.4*±5.1
Aflatoxin M1 (AFM1)	n.d.[2]	0.5*±0.6	4.9±6.4	4.4*±5.2	2.8*±3.5	5.2*±6.2

[1]aflatoxins presence into the vaginal implant: 4.9 mg AFB1, 1.01 mg AFB2, 10.63 mg AFG1 and 0.89 mg AFG2 .

[2]n.d.= not detectable.

Differences with initial value (0 minute) being greater than zero. * $P < 0.05$; ** $P < 0.01$ *** $P < 0.001$.

Figure 11-1. Aflatoxin M1 (AFM1) milk concentration (means ± standard deviation; ng/L) at different milking from aflatoxin contaminated vaginal implant in lactating dairy cows (n=8)