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14 Effects of Mixing and Pelletizing on the Efficacy of a Sequestering Agent in Reducing Aflatoxin M1 Excretion into Milk of Lactating Dairy Cows

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14.1 Abstract

Two experiments were carried out on lactating Holstein cows to determine the effect of different ways of inclusion of sequestering agent on the carry over of aflatoxin B1 (AFB1) from contaminated feeds to milk as aflatoxin M1 (AFM1). Aflatoxin B1 from natural contaminated corn meal (CC) and a pelleted mineral/vitamin protein supplement (PMX) made from contaminated corn meal were utilized. Diets fed to cows were based on corn silage (30%), alfalfa dehydrate hay (25%), grass hay (5%) and ingredients with CC (19%) and PMX (21%). In both experiments cows were fed AFB1 diets for nine days followed by five days of AFB1 free diets. In the first experiment cows were also assigned to four treatments in a replicated 4 x 4 Latin squares design. The sequestering agent was added directly to either the CC (CC-SQ) or the PMX (PMX-SQ) feeds (2.22% and 2.00% of SQ, respectively) or to a contaminated concentrate mix (46.88% CC and 52.07% PMX, 1.05% SQ). The four diets had a common forage source. The AFM1 concentration in milk reached a steady-state condition between the 7th and 9th days from AFB1 starting intake. Sequestering agent inclusion methods affected milk AFM1 concentration and the AFB1 carry over into milk. Higher AFM1 concentrations were observed in diet 3 and diet 4 (111.1 ng/kg and 120.4 ng/kg, respectively) compared to diet 1 (97.3 ng/kg), whereas diet 2 had the lowest AFM1 level (75.7 ng/kg). The lowest carry over (1.3%) was in diet 2 compared to 1.63% of diet 1, 1.95% of diet 3 and 2.03% of diet 4. No differences for carry over were observed between diets 3 and 4. In the second experiment two diets were used: a control diet (CC and PMX) without sequestering agent and diet 1 from experiment one. The presence of the

sequestering agent in diet 1 reduced both the milk AFM1 concentration (47%) and the carry over (44%) as compared to the control diet. Two methods for AFB1 extraction from feeds were tested based either on a methanol/water solution (80:20 vol/vol) or an acetone/water solution (85:15 vol/vol). When sequestering agent was added to feeds the acetone/water solution had a higher AFB1 extraction compared to the methanol/water solution.

Key words: *aflatoxin, carry over, dairy cow, sequestering agent*

14.2 Introduction

Crops such as corn (Zea mays L.), cotton or peanuts and their industrial by products are frequently contaminated by aflatoxins (AFs), hepatocarcinogens molecules (IARC, 2002) produced primarily by Aspergillus flavus and A. parasiticus (Scheidegger and Payne, 2003).

Aflatoxin B1 (AFB1), once ingested by mammals, is rapidly adsorbed in the gastro-intestinal tract and appears as aflatoxin M1 (AFM1), its principal oxidized metabolite, in plasma as soon as fifteen minutes after feeding (Moschini et al., 2007) and in milk as early as the first milking after AFB1 ingestion (Diaz et al., 2004). The AFB1 carry over (CO) into milk as AFM1 has been determined to be around 1-3% and is affected by milk yield and stage of lactation (Veldman et al., 1992; Diaz et al., 2004; Van Eijkeren et al., 2006).

The European Community (EC) allowable limits for AFB1 in animal feeds and concentrates are 20 μg/kg and 5 μg/kg respectively (European Commission, 2003). Furthermore, the EC limits AFM1 in milk to a level not greater than 0.05 μg/L (European Commission, 2006). In the US AFM1 is regulated by the US Food and Drug Administration (FDA) at 0.5 μg/L (Berg, 2003).

The use of sequestering agents (SQ) capable of binding mycotoxins molecules like AFB1 can reduce their absorption in the gastro-intestinal tract and therefore reduce their CO into milk. The addition of SQ is also a popular approach for reducing the negative effects associated with consumption of mycotoxin contaminated feeds (Diaz and Smith, 2005).

Hydrated sodium calcium aluminosilicates (Harvey et al. 1991; Phillips et al. 1988), montmorillonites (Ramos and Hernandez, 1996), bentonites (Schell et al., 2000), zeolites (Piva et al., 1995), activated carbons (Galvano et al., 1996; Diaz et al., 2004), and yeast cell wall extracted glucomannans (Aravind et al., 2003) have been reported to have a high affinity for AFs.

Compared to monogastric animals in which the SQ is normally included to the complete feed (meal or pellet), in lactating dairy cows the SQ is often added during total mixed ration

(TMR) preparation in the mixer wagon, with consequent dilution effect of the SQ respect at AFB1 which could lower the efficiency of the SQ.

One of the most frequently AFB1 contaminated feed in the farm is corn meal, which is normally added during the TMR preparation separately from the SQ. Only when buying a complete feed that includes a SQ would the product be added in advance by the feed mill.

Aflatoxins are highly heat stable (Hwang and Lee, 2006), however, feeds processing like roasting, autoclaving and extrusion-cooking can reduce AFB1 concentration in feeds (Hwang and Lee, 2006; Park and Kim, 2006; Castells et al., 2005). Recently, Oluwafemi (2004) observed a 20% decrease of AFB1 concentration in feeds treated at 100°C for 30 minutes.

No information is currently available on the effects of feed processing on the SQ and AFs interactions. High pressure the pelleting, expansion or extrusion are industrial processes that strongly impact the physical structure of feeds and could potentially have an effect on SQ efficiency.

The objective of this work was to compare the effect of pelleting or simply time of mixing of a commercial SQ either in from a contaminated corn meal or in the a diluted complete concentrate mix on the AFM1 CO into milk of lactating dairy cows.

14.3 Materials and Methods

14.3.1 Feed Preparation

A batch of 3000 kg of AFB1 naturally contaminated corn meal (CC) (32.13 ± 3.38 µg/kg) was obtain from a local producer. A 2000 kg batch of CC was added to 2.22% of the SQ (Atox, Grupo Tolsa, Madrid, Spain), then accurately mixed for three minutes (CC-SQ) in a industrial 3000 kg mixer (MO/30, Grespan, Treviso, Italy).

A mineral/vitamin protein premix (table 14-1), to be used as a separate component in the TMR formulation was prepared (PMX), 2.00% of the SQ was added to half of premix (PMX-SQ), then pelleted after steam conditioning (UMT 1200, Universal Milling Technology, Graz, Austria). The obtained AFB1 basal contamination of premixes was 4.13±0.71 µg/kg.

A 2500 kg batch of an AFB1 complete contaminated concentrate was obtained by mixing 52.07% PMX and 46.88% CC and adding 1.05% of the SQ. Then half of the batch (1250 kg) was pelleted (PC-SQ) after steam conditioning to obtain a 6mm pellet. The reminder of the material was utilized to prepare the MC-SQ that was to be mixed into the TMR formulation.

Feed processing, mixing and pelleting was carried out in a industrial feed mill plant in northern Italy (Ferrari mangimi S.r.l., Sarmato, Italy).

14.3.2 Animals and Diets

Eight multiparous Holstain Friesian cows (mean ± SD) of 604 ± 99 kg of BW, 32 ± 5 kg/d of milk production and 120 ± 22 DIM were utilized in two consequent experimental trials (trial 1 and 2). Trials were carried out at the CERZOO research and experimental center (San Bonico, Piacenza, Italy). The research protocol and animal care was in accordance with the EC council directive guidelines for animals used for experimental and other scientific purpose (European Communities, 1986).

Cows were fed utilizing an electronic gate feeder (American Calan Inc., Northwood, NH) and had free access to water. Animals were milked twice a day (0230 and 1330) and individual milk yield was recorded at every milking (Afimilk system, Afikim, Israel).

Trial 1

Cows were assigned to two balanced 4 x 4 Latin squares. The four diets (diet 1 to 4) fed in the Latin square were formulated according to the nutrient requirements of dairy cattle (NRC, 2001) and are reported in table 14-1. The single components were added in a mixer wagon (Data Ranger, American Calan Inc., Northwood, NH) and mixed for five minutes before distribution. The TMR was fed ad libitum (5% expected orts) once daily (0800). Orts were collected individually and weighted daily.

Each period of the Latin square had a nine days of AFB1 ingestion followed by a seven days of AFM1 clearance from milk periods in which animals were fed the base diet without AFB1 or SQ.

Trial 2

Two weeks after the end of trial 1 the same animals (mean ± SD: 603±102 kg of BW, 32.14 ± 4.91 kg/d of milk production, 185 ± 22 DIM) were randomly assigned to two treatment diets (four cows each) in a completely randomized design. Treatment diets were formulated according to the nutrient requirements of dairy cattle (NRC, 2001) and consisted of a base diet (forage and PMX) with AFB1 contaminated corn meal (CC) in the control diet (CTR) and a diet with contaminated corn meal and SQ (CC-SQ; diet 1) (table 14-1). The TMR preparation,

distribution and orts collection were as in trial 1. The aflatoxin ingestion period lasted nine days followed by a five days of clearance period.

14.3.3 Feed and Milk Sampling

Individual TMR samples and orts were collected at day 0, 3, 5, 7, 9, 11 and 14 for each experimental period. Single feeds entering the TMRs were sampled at day 0 and 7. Samples were dried in a ventilated oven (60°C) for 48 hours, ground with a 1 mm sieve (Thomas-Wiley Laboratory Mill, mod. 4, Arthur H. Thomas Co. Phyladelphia, PA), then analyzed for AFB1 content.

Individual milk samples were collected at each milking at day 0, 3, 5, 7, 9, 11 and 14, proportionally mixed by animal and day, analyzed for fat, protein and lactose content (Milkoscan Model FT120 Foss Electric, Denmark), then frozen at -20°C before AFM1 determination.

14.3.4 Sample Analysis

AFB1 Assay in Feeds

Ten grams of dried feed were mixed in 100 ml of a methanol/water solution (80:20 vol/vol) (Stroka et al., 1999), or in 100 ml of a acetone/water solution (85:15 vol/vol) (Arranz et al., 2006), shaked at 150 rpm for 45 minutes (Universal table Shaker 709) and filtered with Schleicher & Schuell 595 ½ filter paper (Dassel, Germany). Then, 5 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 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 vol/vol) solution and filtered (Millipore Corporation, Bedford, Massachusetts, USA, HV 0.45 μm) before HPLC analysis.

AFM1 Assay in Milk Samples

Extraction was done by the immunoaffinity technique according to Mortimer et al. (1987). Briefly, 50 mL of defatted milk (centrifugated at 7000 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 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 vol/vol) solution and filtered (Millipore Corporation, Bedford, Massachusetts, USA, HV 0.45 μm) before HPLC analysis.

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 AFB1 was separated with a reverse-phase C18 Superspher column (4 μm particle size, 125 x 4 mm i.d.; Merck, Darmstadt, Germany) at room temperature and isocratic conditions, with a mobile phase of water and acetonitrile/methanol solution (17:29 vol/vol) with a 64:36 (vol/vol) 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 365 nm excitation and 440 nm emission wavelengths.

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 vol/vol) mobile phase and the flow rate set at 1.0 mL/min. The fluorescence detector was set at 365 nm excitation and 440 nm emission wavelengths.

The standard stock solutions was checked for AFB1 and AFM1 concentration according to A.O.A.C. method 970.44 (AOAC, 1995) and stored at -20°C when not in use.

14.3.5 Carry Over Calculation

The total excretion and CO of AFB1 into milk as AFM1 were obtained based on individual AFB1 daily intake, milk yield and AFM1 concentration. The days considered for calculation were at plateau condition, between 7th and 9th days on treatment.

14.3.6 Statistical Analyses

Data from trial 1 were analyzed by mixed procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) as a 4 x 4 Latin square design, replicated two times, using the following model:

$$Yijkl = \mu + Di + Sj + Cl(j) + Pk + eijkl$$

where Yijkl = dependent variable (DMI, milk, fat, protein and lactose yield, AFB1 intake, AFM1 concentration and carry over), μ = overall mean, Di = mixed effect of the diet (i = 4), Sj = mixed effect of the square (j = 2), Ci(l) = random effect of cow (l = 4) nested within square i, Pk = mixed effect of the period (k = 4); and eijkl = residual error. Least squares means were separated into significant main effects by the PDIFF option of SAS. DMI, milk, fat, protein and lactose yield, AFB1 intake, AFM1 concentration and carry over data were analyzed as repeated measures in time using the MIXED procedure of SAS (Littell et al., 1996). The experimental unit was the cow.

Data from trial 2 were analyzed by the general linear model procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC) as a complete randomized design with the single cow as experimental unit. Pair wise comparisons among means were performed using T test. Differences were declared significant for $P < 0.05$.

14.4 Results

Analytical Assay of Feeds. The AFB1 extraction methods used for feeds analysis had considerable effects on final AFB1 amount when the SQ was added to the feed. Lower AFB1 extractions were observed when using the methanol/water compared to the acetone/water solvent solution (table 14-2). The discrepancy between the two methods was 72.1, 80.0 and 87.0%, respectively for MC-SQ, PC-SQ and CC-SQ feeds. Similar AFB1 values between methods were observed mixed concentrates.

14.4.1 In vivo Experiment

The average AFB1 contamination of the basal diet was 0.68 ± 0.04 μg/kg which contributed to a bulk milk AFM1 content of 13.7 ± 5.1 ng/kg.

Trial 1

Reported results on AFB1 contents of diets were obtained with the acetone/water solution extraction method (table 14-3). The calculated AFB1 intake for each cow was 171.3 μg/d.

The way of the SQ addition to feeds, pelleted or not, affected ($P < 0.05$; table 14-3) the milk AFM1 concentration (ng/kg) and the AFB1 carry over (%) to milk as AFM1. The AFM1 concentration decreased (22%; $P < 0.05$) in diet 2 whereas increased (14 and 24%; $P < 0.05$) respectively for the diets 3 and 4 compared to diet 1. No differences among groups were reported on AFB1 and dry matter intake or in milk yield. The lowest carry over (1.3%) was measured for diet 2, significantly lower ($P < 0.05$) than diet 1 (1.63%), 3 (1.95%) and 4 (2.03%); no differences were observed between diets 3 and 4.

Trial 2

Cows received 173.9 µg/d of AFB1 as average. The presence of the SQ mixed with the contaminated corn (2.22%) reduced ($P < 0.05$; table 14-4) the milk AFM1 concentration (47%) and the AFB1 carry over (44%) to milk as AFM1 compared to a control AFB1 contaminated diet (CTR) with no SQ addition. No differences were observed on AFB1 and dry matter intake and milk yield.

14.5 Discussion

14.5.1 Analytical Assay of Feeds

The different AFB1 values on SQ added feeds observed when using methanol or acetone extraction procedure can be misleading. In particular, the same feed could comply with the 5 µg/kg threshold, as the maximum AFB1 content allowed by the EU legislation in mixed feeds for animal use (European Commission, 2003), when analyzed for AFB1 content using the methanol/water extraction solution but at the same time could be over the limit when analyzed with the acetone/water extraction solution. Results suggest that the SQ when added to AFB1 contaminated feeds can reduce the AFB1 concentration to a level below the threshold value when the methanol/water extraction solution is being used. Also, the binding capacity of SQ seems not to have been changed by the physical processing of the feeds with the level of contamination utilized. Data outline that in feed analysis it is of paramount importance to know if the reported AFB1 contamination level was obtained in sequestering agent added feeds.

If the methanol/water solution is good for the AFB1 extraction in concentrate, the same analytical procedure seems inadequate to our experimental condition.

14.5.2 In vivo Experiment

The method in which the SQ was added to the diet affected the AFB1 carry over and the AFM1 concentration in milk (table 14-3; figure 14-1). The less effective way of counteracting the aflatoxin presence in feeds was observed when the SQ was added to the mineral/vitamin protein premix (PMX-SQ), and then to the base diet with the contaminated corn meal (diet 4). The measured AFB1 carry over was 2.03%. The low performance of the SQ could be caused by the physical separation between the sequestering agent, within the pellet of the premix, and AFB1 in the contaminated corn meal. However, diet 3 characterized by the concurrent presence of contaminated corn meal and the SQ and no pellet processing did not improve the AFB1 carry over and the total AFM1 in milk compared to the diet 4. Thus, in our conditions the physical contact between contaminated corn meal and SQ might not fully explain the SQ performance. Additionally, the pellet itself (diet 4) did not act as a time delay factor for SQ efficiency within the gastro-intestinal tract. Diet 3 represented the normal procedure for the SQ utilization on a commercial dairy farm.

The best performance was obtained in diet 2, which was similar to diet 3, except for a pelleting processing leading to a complete concentrate pellet containing the SQ and the contaminated corn meal. Under this processing condition the concentrate was steam conditioned to 80°C and 18% moisture (plus 6% the standard 12% moisture content of the corn meal), then pressure was increased during the pelleting processing up to a final specific weight of 0.713 (wt/vol). From our result it was clear that the physical processing was improving the strength of AFB1/SQ interactions.

The intermediate results obtained in diet 1 could have different implication. Even though the final content of the SQ in diets was similar, in terms of feeds preparation the dilution factors (aflatoxin and sequestering agent) were different. The diet 1 was obtained by adding to the same base diet the same amount of contaminated corn meal with double of the percentage content of the SQ. Thus, the result seemed to support the idea of a dilution factor that could improve the effect of the SQ.

If contact time between the AFB1 and the sequestering agent is the only factor affecting the binding performance, then we could think of that time as being spread between silo and rumen compartments. Giving a fixed rumen flow rate, the presence of a pellet compared to a meal could affect the time needed for rumen escaping of feed particles. This could in a way increase the time length of the SQ within the gastro-intestinal tract. However, results of diets 3 and 4 did not support this hypothesis. From our results it was impossible to prove the effect of time of a close contact between the SQ and AFB1 outside the rumen (silo compartment), however, the

dilution factor (diet 1) seemed more important than the time length within the rumen (diet 3) as by the AFM1 concentration and the AFB1 CO observed in both diets. The two diets had similar SQ content, however, there was a different SQ content mixed in the corn meal. The ratio between SQ and AFB1 in corn meal were 700 and 325 (wt/wt), respectively for diet 1 and 3.

Even though the dilution factor proved to be important for SQ performance compared to the normal sequestering agent usage in farm condition (diet 3), the effect was lower than the pelleting processing observed in diet 2 with half the dilution factor. These results underline the importance of processes occurring during the physical treatment leading to the conclusion that the pelleting step could be an important way of improving the SQ performance.

Results on calculated carry over could have been different according to the methods used for AFB1 determination of contaminated diets. Based on what observed on AFB1 feeds analysis when the two methods were used (table 14-2) it was clear a lack of the methanol/water solution to extract the AFB1 in presence of SQ. The result itself would not affect the amount of AFM1 getting into the milk, however, the ultimate result could be critical when complying with the maximum level of AFM1 into milk. The AFM1 content of milk from treated groups were all above the maximum EU limit of 0.05 µg/L (European Commission, 2006).

In trial 1 the estimated AFB1 intake (µg/cow/d) based on the methanol/water method was 39.49, 32.97, 45.03 and 149.3 respectively for diet 1, 2, 3 and 4. Considering a 1.5% carry over value of AFB1 into milk as AFM1 we could expect diets 1 to 3 to comply with the EC limit for AFM1 in milk. Then, diets would be considered legal and only diet 4, with the SQ not mixed directly with the contaminated corn meal, would be considered at risk and potentially blocked at the plant. However, the results from trial 1 proved this was not the case, with all diets leading to milk AFM1 concentrations over the allowed EC limit (table 14-3). Based on the estimated AFB1 intake (with the methanol/water method), the real total AFM1 in milk would justify carry over of 7.2, 6.9, 7.4 and 2.4%, respectively for diets 1, 2, 3 and 4; well above any reported except for diet 4. The calculated carry over from AFB1 intake estimated from value obtained with acetone/water extraction (table 14-3) was in agreement with previous work (Veldman et al., 1992; Diaz et al., 2004; Masoero et al., 2007).

It was clear from our data that only one of the analytical methods utilized for feed analysis in presence of SQ was as effective. Thus, when using the methanol/water extraction solution a competitive reaction between SQ and the organic solvent versus the AFB1 would benefit the SQ, though it would be later be released during the digestive processes.

The hypothesis of a weak extraction efficiency of organic solvents in presence of a clay type SQ can be considered observing the demonstrated efficiency smectite clay SQ in binding the

AFB1 in the gastro-intestinal tract of poultry (Pimpukdee et al., 2004) and swine (Phillips et al., 1988; Schell et al., 2000) and dairy cows (Harvey et al., 1991; Diaz et al., 2004). Also, previous data from our lab (M. Moschini, unpublished data) showed a different tendency of several adsorbents to release adsorbed AFB1 during the digestive processes.

Data from trial 2 showed that a SQ can be effectively utilized to reduce the CO of AFB1 into milk on dairy cows. The diet containing SQ was the same as diet 1 of trial 1 and it was chosen as alternative diet because of its performance in trial 1 and because it was not pelleted. The AFM1 concentration in milk of both groups consuming the same experimental diets was 97.3 ng/kg trial 1 (table 14-3) and 113.1 ng/kg in trial 2 (table 14-4) with an overlapping 0.95% confidence interval.

The AFM1 content in milk from cows fed the CTR was 215.1 ng/kg (table 14-4) very similar to 208.96 ng/kg calculated utilizing the Veldman et al. (1992) equation with an AFB1 intake of 174.00 µg/d.

14.6 Conclusion

The method utilized during SQ addition had a significant effect in the ability of the SQ agent to reduce the AFM1 carry over into milk. Additionally, it was clear that the physical processing method had an effect in improving the amount of AFB1 being sequestered by SQ. In addition, results supported the idea that a dilution factor can improve the effect of SQ in reducing the AFB1 available for absorption in the gastro-intestinal tract. The dilution factor proved to be important for SQ performance, however, the contribution seemed to be lower than that seen after pelletting. Results from our trials underline the need of an AFB1 extraction methodology working similarly in feeds either added or not different SQ to avoid negative drawback in farm condition.

Table 14-1. Ingredients (% of DM) of the five experimental diets (trial 1 and 2).

	Diets				
Ingredients (% DM)	CTR	diet 1	diet 2	diet 3	diet 4
Corn silage	30.0	30.0	30.0	30.0	30.0
Alfalfa hay, dehydrate	25.0	25.0	25.0	25.0	25.0
Grass hay	5.0	5.0	5.0	5.0	5.0
CC	19.0				19.0
CC-SQ[2]		19.0			
PMX[1]	21.0	21.0			
PMX-SQ[3]					21.0
MC-SQ[4]				40.0	
PC-SQ[4]			40.0		

[1]Contains per kg of premix: Soybean meal 600 g, Sunflower meal 300 g, mineral and vitamin supplement 100 g.; 120000 IU of Vitamin A; 9000 IU of Vitamin D3; 90 mg of Vitamin E; 3.6 mg of Co; 19.2 mg of I; 1.44 mg of Se; 600 mg of Mn; 62.4 mg of Cu; 2240 mg of Zn; 1.92 mg of Mo; 360 mg of Fe.

[2]2.22% of the SQ.

[3]2.00% of the SQ.

[4]1.05% of the SQ.

[5]CC: naturally contaminated corn meal; CC-SQ: CC + 2% Atox®; PMX: mineral vitamin premix; PMX-SQ: PMX + 2% Atox®; MC-SQ: 55% PMX + 45% CC + 1% Atox®; PC-SQ: 55% PMX + 45% CC + 1% Atox® then pelleted.

Table 14-2. Aflatoxin B1 (AFB1) concentration (mean ± SD; µg/kg) in feeds used in trial 1 and 2 as obtained from two different extraction (methanol/water and acetone/water solutions).

Ingredients	n[1]	Methanol/water solution	Acetone/water solution
CC	8	30.60 ± 10.63	32.13 ± 3.38
CC-SQ	8	4.09 ± 2.31	31.47 ± 5.08
PMX	8	4.13 ± 0.71	5.64 ± 0.45
PMX-SQ	8	1.15 ± 1.43	3.82 ± 0.32
MC-SQ	8	4.74 ± 1.04	17.00 ± 0.45
PC-SQ	8	3.46 ± 0.70	17.33 ± 0.66
CC-PMX	16	17.16 ± 3.99	18.20 ± 1.62

[1]n = total number of analytic replicates.

[5]CC: naturally contaminated corn meal; CC-SQ: CC + 2% Atox®; PMX: mineral vitamin premix; PMX-SQ: PMX + 2% Atox®; MC-SQ: 55% PMX + 45% CC + 1% Atox®; PC-SQ: 55% PMX + 45% CC + 1% Atox® then pelleted.

Table 14-3. Trial 1: Mean of DIM (kg/d), milk production (kg/d), fat, protein and lactose (g/kg), aflatoxin B1 (AFB1) intake (µg/d), aflatoxin M1 (AFM1) milk concentration (ng/kg) and carry over in milk (%) at plateau condition (7th and 9th day on AFB1 ingestion period).

Item	Diets				SE
	diet 1	diet 2	diet 3	diet 4	
DMI, kg/d	23.06	22.73	23.09	23.42	0.2680
Milk, production kg/d	29.29	30.09	29.93	29.37	0.4173
Fat, g/kg	3.78	3.66	3.67	3.78	0.0884
Protein, g/kg	3.41	3.39	3.44	3.43	0.0291
Lactose, g/kg	5.10	5.12	5.10	4.99	0.0727
AFB1 intake, µg/d	172.42	170.42	170.39	171.85	0.7239
AFM1, ng/kg	97.3[b]	75.7[a]	111.1[c]	120.4[c]	3.6513
Carry over, %	1.63[b]	1.30[a]	1.95[c]	2.03[c]	0.0675

[a-c] Means within a row with different superscript differ ($P < 0.05$).

[5]CC: naturally contaminated corn meal; CC-SQ: CC + 2% Atox®; PMX: mineral vitamin premix; PMX-SQ: PMX + 2% Atox®; MC-SQ: 55% PMX + 45% CC + 1% Atox®; PC-SQ: 55% PMX + 45% CC + 1% Atox® then pelleted.

Table 14-4. Trial 2: Mean of DIM (kg/d), milk production (kg/d), aflatoxin B1 (AFB1) intake (µg/d), aflatoxin M1 (AFM1) milk concentration (ng/kg) and carry over in milk (%) at plateau condition (7th and 9th day on AFB1 ingestion period).

Item	Diet		SE	P-value[1]
	CTR	Diet 1		
DMI, kg/d	23.80	23.27	0.8581	0.6704
Milk, production kg/d	31.03	33.25	1.7470	0.3831
AFB1 intake, µg/day	174.00	173.79	0.3411	0.6699
AFM1, ng/kg	215.1[a]	113.1[b]	6.2708	<0.001
Carry over, %	3.81[a]	2.14[b]	0.1386	<0.001

[a,b] Means within a row with different superscript differ ($P < 0.05$).

[1] *P*-value…

CC-PMX: naturally contaminated corn meal + mineral vitamin premix

CC-SQ: CC + 2.22% Atox®

Figure 14-1. AFM1 concentration (ng/kg) in the milk of cows from different diets: (■) diet 1, (□) diet 2, (▲) diet 3, and (Δ) diet 4. For each diets the point represents the mean of 8 data.