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Carryover of aflatoxin from feed to milk in dairy cows with low or high somatic cell counts

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Aflatoxin M1 (AFM1) residues in milk are regulated in many parts of the world and can cost dairy farmers significantly due to lost milk sales. Additionally, due to the carcinogenicity of this compound contaminated milk can be a major public health concern. Thirty-four lactating dairy cows were utilised to investigate the relationship between somatic cell counts (SCC), milk yield and conversion of dietary aflatoxin B1 (AFB1) into milk AFM1 (carryover (CO)). The AFM1 in milk increased as soon as the first milking after animal ingestion with a pattern of increment up to the observed plateau (between 7th and 12th days of AFB1 ingestion). There was a significant ($P < 0.01$) effect of the milk yield whereas no effect could be attributed to the SCC levels or to the milk yield × SCC interaction. Similarly, the main effect of milk yield was also observed ($P < 0.01$) on the total amount of AFM1 excreted during the ingestion period. Although the plasma concentration of gamma-glutamyl transferase was significantly affected by aflatoxin administration, levels of this liver enzyme were within the normal range for lactating dairy cows. The current data suggest that milk yield is the major factor affecting the total excretion of AFM1 and that SCC as an indicator of mammary gland permeability was not related to an increase in AFM1 CO.

Keywords: aflatoxins, aflatoxin M1, dairy cows, milk, somatic cell count

Introduction

The aflatoxins are secondary metabolites produced primarily by *Aspergillus flavus* and *A. parasiticus.* Aflatoxins are common crop contaminants, with contamination occurring in the field, during harvest or during storage. The most frequently affected crops are maize (*Zea mais*), cotton and peanuts and their by-products. The major aflatoxins are aflatoxin B1 (AFB1), B2, G1 and G2. Because of their low molecular weight, once ingested these compounds are rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism (Yiannikouris and Jouany, 2002) and quickly appear as a metabolite in blood after just 15 min (Moschini *et al.*, 2006) and in milk as soon as 12 h post-feeding (Diaz *et al.*, 2004).

Aflatoxin M1 (AFM1) is the principal oxidised metabolite of AFB1 and can be readily found in the milk and urine of most mammalians after consumption of AFB1. The aflatoxins, as a group (AFB1, AFB2, AFG1, AFG2 and AFM1), are classified as group 1 carcinogens (International Agency for Research on Cancer, 2002). The European Union allowable limits for AFB1 in animal feeds and concentrates are 20 and 5 μg/kg, respectively (European Community, 2003). Furthermore, the European Community (EC) limits AFM1 in milk to levels not greater than 0.05 μg/l (European Community, 2006). In the US, AFM1 is regulated by the US Food and Drug Administration (FDA) at 0.5 μg/l.

In dairy cows the amount of AFM1 excreted into milk can be up to 3% of the AFB1 intake (Diaz *et al.*, 2004) and is affected by milk yield (Pettersson *et al.*, 1989; Veldman *et al.*, 1992) and stage of lactation (Munksgaard *et al.*, 1987; Pettersson *et al.*, 1989; Veldman *et al.*, 1992). Other factors that affect carryover (CO) into milk include species differences (Battacone *et al.*, 2003), animal variability (Van Egmond, 1989; Steiner *et al.*, 1990; Veldman *et al.*, 1992) and mammary alveolar cell membrane health (Lafont *et al.*, 1983).

There is limited information about the effect of udder infection on AFM1 excretion into milk. It has been suggested that an increase in AFM1 CO occurs due to *Staphylococcus* udder infection (Veldman *et al.*, 1992) whereas a previous study showed a relationship between AFM1 milk CO and milk somatic cell counts (SCC) independent of the

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milk yield (Lafont *et al*., 1983). Mastitis increases the number of somatic cells in milk, alters milk composition (Walstra and Jenness, 1984) and may affect AFM1 CO rate by increasing membrane permeability.

The objective of this work was to evaluate the effect of milk yield and SCC, as indicators of udder inflammatory processes, on milk AFM1 CO in lactating dairy cows.

Material and methods

Animals and treatments

An experiment was carried out utilising 34 Holstein multiparous cows housed at the CERZOO research and experimental centre (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 purposes (European Community, 1986).

Milk yield and SCC data for classification of animals as high or low were obtained as the average of three measurements during a 15-day pre-experimental period. Individual milk yield for the factorial arrangement was measured daily during the last week of the pre-experimental period. Milk yield (LY: $<$ 30 kg per head per day; HY: $>$ 30 kg per head per day) and milk SCC (LSCC: $<$ 350 000; HSCC: $>$ 350 000) were used in a 2 $\times$ 2 factorial arrangement in a completely randomised design.

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 (National Research Council, 2001) for an average cow weighing 600 kg, 140 days in milk and a 35 kg milk yield (3.8% fat and 3.35% protein). The bulk of the diet (Table 1) on a dry-matter basis was maize silage (31.2%), dehydrated alfalfa hay (16.7%), grass hay (4.1%) and energy-protein supplement (48%). The diet was fed *ad libitum* (5% expected orts) as a total mixed ration (TMR) daily (0900 h). Cows were milked twice a day (0230 and 1330 h) and individual milk yield was recorded at every milking (Afimilk system, Afikim, Israel).

During the experimental period (10 days), cows were given, before the morning meal, a 300 g bolus containing 1.004 $\pm$ 0.03 g per cow per day of a naturally contaminated corn meal. This allowed for an AFB1 intake from the contaminated bolus of 98.10 $\pm$ 0.26 μg per cow per day.

TMR samples were collected on days 0 and 10 of the experimental period, dried at 55°C in a ventilated oven until constant weight, and then ground with a 1-mm sieve (Thomas-Wiley Laboratory Mill, Arthur H. Thomas Co., Philadelphia, PA, USA) and frozen until aflatoxin analysis.

Individual milk samples were collected at each milking for 18 consecutive days (during the last 2 days of the pre-experimental period, the experimental period (10 days) and for 6 days at the end of the AFB1 ingestion period); then a representative sample for day of milking was obtained and stored at $-$18°C for subsequent analysis. Milk samples collected at days 3, 7 and 10 of the treatment period were analysed for fat, protein and lactose contents (infrared analysis, Milkoscan Model FT120 Foss Electric, Hillerod, Denmark) and for SCC (Fossomatic 360 Foss Electric, Hillerod, Denmark).

Table 1 *Ingredients and chemical composition of the basal diet*

	Base diet
Ingredients (g/kg dry matter (DM))	
Maize silage	312
Alfalfa hay, dehydrate	167
Grass hay	41
Cotton seed, whole with lint	85
Maize meal	183
Barley meal	66
Protein supplement[†]	103
Calcium soap (Megalac)	9
Soya-bean meal	34
Chemical composition (g/kg DM)	
Crude protein	162
Crude lipids	49
ADF[‡]	204
NDF[‡]	340
Calculated	
PeNDF[§]	267
NSC[§]	412
Forage (%)	52
Net energy lactation (MJ/kg DM)[¶]	7.08

[†]Contains per kg of pre-mix: soya-bean meal 600 g, sunflower meal 300 g, mineral and vitamin supplement 100 g; 120 000 IU of vitamin A; 9000 IU of vitamin D_3; 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.

[‡]ADF = acid-detergent fibre expressed without residual ash. NDF = neutral-detergent fibre according to Van Soest *et al*. (1991) without sodium sulphite and with alpha-amylase; expressed without residual ash.

[§]PeNDF = physical effective neutral-detergent fibre (Mertens, 1997), calculated according to the contribution of the single feed present into the diet (concentrates were considered with PeNDF = 0; whole cotton seeds PeNDF = 70). NSC was calculated as NSC = 100 $-$ (NDF + ASH + crude protein + crude lipids).

[¶]According to NRC (2001).

Sample analysis

AFB1 assay in feeds. Ten grams of dried feed were put in a methanol : water solution (80 : 20 vol/vol), shaken at 150 r.p.m. for 45 min (Universal Table Shaker 709) and filtered with Schleicher & Schuell 595 ½ filter paper (Dassel, Germany). Then, 5 ml were eluted with 45 ml of bidistilled water through an immunoaffinity column (Aflatoxin Easi-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, MA, USA; HV 0.45 μm) before HPLC analysis.

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AFM1 assay in milk samples. Extraction was carried out by the immunoaffinity technique, according to Mortimer *et al*. (1987). Briefly, 50 ml of defatted milk (centrifuged at 7000 r.p.m. for 10 min 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) solution and filtered (Millipore Corporation, Bedford, MA, 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 an 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 × 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 1 ml/min. Then, the AFB1 was detected by fluorescence, after post-column dramatisation (Jasco 2080 Plus HPLC pump) with pyridinium hydrobromide perbromide (PBPB) at a flow of 0.1 ml/min. The fluorescence detector was set at 365 nm excitation and 440 nm emission wavelengths. The standard stock solution was checked for AFB1 concentration according to AOAC method 970.44 (Association of Official Analytical Chemists (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 × 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 ml/min. The fluorescence detector was set at 365 nm excitation and 440 nm emission wavelengths. The standard stock solution was checked for AFM1 concentration according to AOAC method 970.44 (AOAC, 1995) and stored at −20°C when not in use.

Blood samples

Blood samples were taken before the morning meal via jugular venipuncture on days 0 and 10 of the experimental period. The blood was collected into Li-heparinised (17 U of heparin per ml of blood) Vacutainer (Vacutainer systems, Belliver industrial estate, Plymouth, UK). Then, plasma was obtained by centrifugation (3000 r.p.m. for 15 min). The plasma fraction was isolated and stored at −20°C until analysed for albumin, globulin, aspartate aminotransferase, gamma-glutamyl transferase and bilirubin and beta-hydroxybutyrate concentrations (Bertoni, 1999).

CO calculation

The CO of AFM1 in milk was calculated as the percentage of the AFB1 consumed that was excreted as AFM1 in milk at the time when the toxin output in milk reached a steady state.

Statistical analyses

The AFB1 intake, AFM1 milk concentration, and total excretion and the CO in milk were analysed using the mixed procedure of SAS® (Statistical Analysis System Institute, 2001). A factorial arrangement was used, and fixed effect in the model included the milk yield and the milk SCC. The animal, within-milk yield, and SSC interactions were included as random effects. The day of collection was a repeated measure (compound symmetry covariance structure).

The CO (%) was regressed on milk yield (kg) over time of collection (day) and the linear equation was calculated.

Plasma parameters (pre-experimental and after AFB1 ingestion period) were compared using the paired *t*-test (Statistical Analysis System Institute, 2001).

Results

As expected, the low AFB1 concentration fed to animals did not cause negative health problems during the experimental period. The initial milk yield and the milk SCC for groups were (mean ± s.d.) 21.2 ± 3.8 and 127 600 ± 161 203, 21.7 ± 3.9 and 1 171 889 ± 676 859, 41.8 ± 8.4 and 240 000 ± 20 075, and 34.8 ± 4.6 and 2 030 667 ± 2 451 213, respectively, for LY-LSCC, LY-HSCC, HY-LSCC and HY-HSCC.

The TMR had a base AFB1 content of 3.70 ± 0.21 μg/kg contributing to a bulk milk AFM1 content of 4.80 ± 1.80 and 3.90 ± 1.72 ng/l, respectively, before and after the ingestion period.

The AFM1 in milk increased as soon as the first milking after animal ingestion with a pattern of increment up to the observed plateau (between the 7th and 12th days of ingestion) as reported in Figure 1. At day 1 of AFB1 ingestion, the AFM1 contents in milk were 52.9 *v.* 24.9 and 44.0 *v.* 39.3 ng/l, respectively, for the HY-HSCC, HY-LSCC, LY-HSCC and LY-LSCC groups. Closer values among groups were observed from the 3rd day until the end of the ingestion period with average AFM1 contents at the plateau conditions of 65.8, 61.9, 66.7 and 59.2 ng/l respectively for the HY-HSCC, HY-LSCC, LY-HSCC and LY-LSCC groups (Table 2).

The AFB1 CO into milk calculated at the plateau was 2.32, 2.70, 1.48 and 1.29% of the AFB1 consumed, respectively, for the HY-HSCC, HY-LSCC, LY-HSCC and LY-LSCC groups. There was a significant ($P < 0.01$) effect of

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the milk yield whereas no effect could be attributed to the somatic cell count level or to the milk yield × somatic cell count interaction. Similarly, the main effect of milk was also observed ($P < 0.01$) on the total amount of AFM1 excreted during the AFB1 ingestion period.

Plasma biochemistry for samples collected before and after the aflatoxin ingestion period are reported in Table 3.

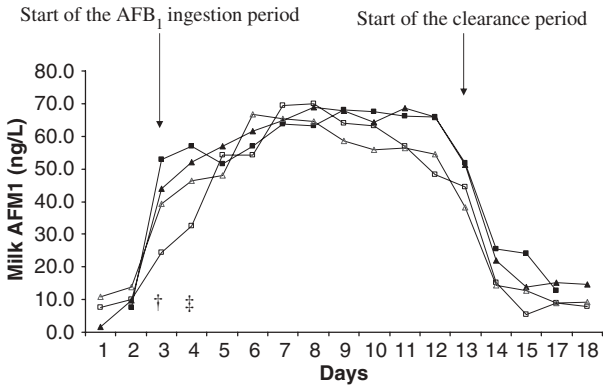

Figure 1 AFM1 concentration in the milk of cows from different factorial arrangement: (■) HY-HSCC, (□) HY-LSCC, (▲) LY-HSCC, (△) LY-LSCC. †Somatic cells count effect ($P < 0.01$). Production and somatic cells count interaction effect sliced for the level of production significant ($P < 0.01$) in the HY group. ‡Somatic cells count effect ($P < 0.05$). Production and somatic cells count interaction effect sliced for the level of production significant ($P < 0.05$) in the HY group.

Discussion

The presence of AFM1 in milk was detectable from the first milking after the animal AFB1 ingestion (Figure 1), which is in accordance with previous work (Allcroft *et al.*, 1968; Trucksess *et al.*, 1983; Diaz *et al.*, 2004). In particular, in the early stage of increase of the AFM1 plateau (between the 7th and 12th days of ingestion) there was an effect ($P < 0.01$) of the SCC. However, the SCC effect was confined to the high-yield groups ($P < 0.01$ and $P < 0.05$, respectively, for the 1st and 2nd day of ingestion) due to the milk yield and SCC interaction being separated from the level of production (Figure 1). Results suggest that high milk yield could intensify the effect of SCC on AFM1 CO. A previous report suggested a positive correlation between SCC and milk AFM1 content in dairy cows fed an AFB1-contaminated diet (Lafont *et al.*, 1983). However, these authors did not report the effect of milk yield in their study.

The observed plateau developed later than in previous studies, in which the steady state for AFM1 was established at 24 (Frobish *et al.*, 1986) and 76 h (Polan *et al.*, 1974, Diaz *et al.*, 2004) from the initial AFB1 ingestion, but is in agreement with results reported by Battacone *et al.* (2003) on sheep, in which the observed plateau condition was between day 9 and day 13 from the first AFB1 ingestion. However, milk AFM1 levels in the present study were lower than previously reported maximum concentrations. Previous studies with dairy cows report a plateau at maximum AFM1 concentrations in milk (Polan *et al.*, 1974; Frobish *et al.*, 1986; Pettersson *et al.*, 1989).

Table 2 *AFB1 intake (μg), AFM1 milk concentration (ng/l) and total excretion (μg per cow per day) and carryover (%) at plateau (7th to 12th day on AFB1 ingestion)*

Item	Groups				s.e.	Main effects ($P<$)		
	HY-HSCC	HY-LSCC	LY-HSCC	LY-LSCC		Milk yield	SCC	Milk yield × SCC
AFB1 intake (μg)	98.0	98.3	98.0	98.1	0.064	0.455	0.007	0.141
AFM1 (ng/l)	65.8	61.9	66.7	59.2	4.908	0.915	0.500	0.832
Total AFM1 excreted (μg per cow per day)	2.27	2.66	1.45	1.27	0.177	0.001	0.756	0.360
Carryover (%)	2.32	2.70	1.48	1.29	0.180	0.001	0.767	0.366

Table 3 *Blood parameters for cows with low somatic cell counts (LY-LSCC and HY-LSCC) before and after AFB1 ingestion*†

Parameter	Before ingestion	After ingestion	s.e.	P‡	Range§
Albumin (g/l)	33.75	37.50	0.871	0.0012	32.3–35.9
Globulin (g/l)	33.83	38.50	1.469	0.0088	38.3–57.6
Aspartate aminotrasnferase (U/l)	63.17	68.25	3.903	0.2194	61.1–103
Gamma-glutamyl transferase (U/l)	26.58	29.58	0.739	0.0019	20.8–45.1
Bilirubin (μmol/l)	2.78	2.56	0.272	0.4261	1.63–4.58
Beta-hydroxybutyrate (mmol/l)	0.51	0.46	0.082	0.5357	0.16–0.75

†Cows consuming a 300 g bolus containing 1.004 ± 0.03 g per cow per day of a naturally contaminated maize meal. This allowed for an AFB1 intake from the contaminated bolus of 98.10 ± 0.26 μg per cow per day.
‡For (after–before) being different than zero.
§Bertoni (1999).

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The AFB1 is promptly absorbed within the gastrointestinal tract of dairy cows and rapidly transferred as AFM1 into milk (Polan *et al.*, 1974). Milking cows fed a 5 mg AFB1 bolus had detectable blood plasma AFM1 and AFB1 concentrations as soon as 15 min after treatment, indicating both a rapid absorption of AFB1 through the rumen wall and metabolism into AFM1 (Moschini *et al.*, 2006). The authors in this study used the retinol palmitate plasma level as a marker for intestinal adsorption (Bertoni *et al.*, 2001), which indicated a probable AFB1 absorption at the rumen level and an intestinal contribution to the AFM1 plasma level 120 min after drenching.

The adsorption and consequent transfer to blood and biological fluids 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. Because of their low molecular weight (AFB1 = 312.27 and AFM1 = 328.27 formula weight), the toxins are rapidly adsorbed through membranes by a passive mechanism (Yiannikouris and Jouany, 2002). Upon adsorption, the body's ability for AFB1 detoxification is associated with the action of the liver microsomal cytochrome *P*-450 enzyme family and the enzyme *S*-glutation-transferase (Galtier, 1999). This system is effective within 7 and 351 μg per head per day (Munksgaard *et al.*, 1987). The level of AFB1 being used in our trial was lower than 80 μg/kg BW, a threshold value after which a decrease of feed intake was observed in calves (Lynch *et al.*, 1971).

Milk yield was decreased when feeding 100 μg/kg AFB1 (Patterson and Anderson, 1982), and a considerable milk yield reduction was observed in cows fed 100 and 300 μg AFB1 per kg BW (Mertens and Wyatt, 1977). Similar results were obtained by Applebaum *et al.* (1982). The level of AFB1 contamination used in our trial (0.16 μg/kg BW) was lower than the indicated threshold value of 100 μg/kg BW for milk yield depression, and no changes in milk yield pattern were observed during the AFB1 ingestion period, as would be expected (Figure 2).

Several factors could affect aflatoxin CO. The variability observed among animals could be related to differences in rumen degradation activity (Westlake *et al.*, 1989), difference in rumen biotransformation to aflatoxicol and other metabolites other than AFM1 (Auerbach *et al.*, 1998), differences in terms of induction of the enzymatic AFB1 oxidation system (Steiner *et al.*, 1990) and differences in mammary gland permeability (Lafont *et al.*, 1983).

Milk AFM1 content has been previously related to SCC (Lafont *et al.*, 1983). However, more recent work suggests that milk yield is the main factor contributing to the total AFM1 excretion (Pettersson *et al.*, 1989; Veldman *et al.*, 1992). In our trial, the total AFM1 excretion and the CO in milk were affected by the milk yield and not by the SCC during the plateau period (Table 2). These data suggest a higher AFM1 excretion in high-milk-yield cows compared with low-milk-yield cows.

Under the conditions of this experiment, in which all cows received AFB1, and the levels of AFB1 exposure, the relationship between CO (%) and milk yield (kg) can be described as follows (Figure 3):

$$\text{CO} = -0.326 + 0.077 \times \text{milk yield}$$
$$(\text{residual s.d.} = 0.692;\ R^2 = 0.58).$$

The distribution of the residuals outlines the absence of any bias related to the milk yield on CO estimate. On average, the high-milk-yielding cows had a 1.81-fold increase in the CO to milk, which is in agreement with previous reported data in early and late lactating dairy cows (Lafont *et al.*, 1983; Veldman *et al.*, 1992). The inappropriate implementation of equations relating CO and milk yield could lead to erroneous conclusions in terms of maximum AFB1 daily intake to comply with the EU limit of AFM1 in milk. For instance, the plotting of the estimated CO obtained when applying the Veldman *et al.* (1992) equation to our milk yield against values calculated with our equation clearly indicate the presence of factors not accounted for (source of contamination, animal variability, etc.) in both equations, which limits the equations to their respective trials for CO estimates (Figure 4). A simple steady-state model has been proposed for the disposition of AFB1 and AFM1 in the lactating cow (Van Eijkeren *et al.*, 2006). The

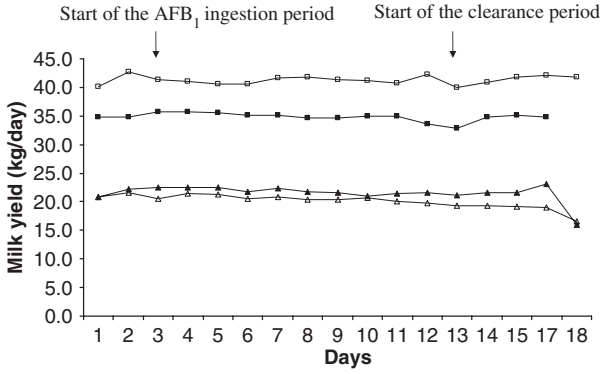

Figure 2 Milk yield of cows from different factorial arrangement: (■) HY-HSCC, (□) HY-LSCC, (▲) LY-HSCC and (△) LY-LSCC.

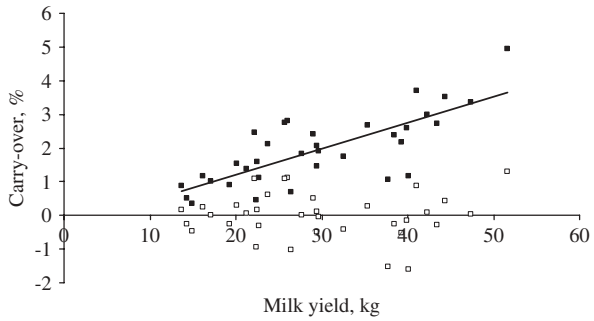

Figure 3 Plot of observed (■) and residuals (□) for carryover *v.* milk yield (kg). Carry over = −0.326 + 0.077 × milk yield (kg) (residual s.d. = 0.692, $R^2 = 0.58$).

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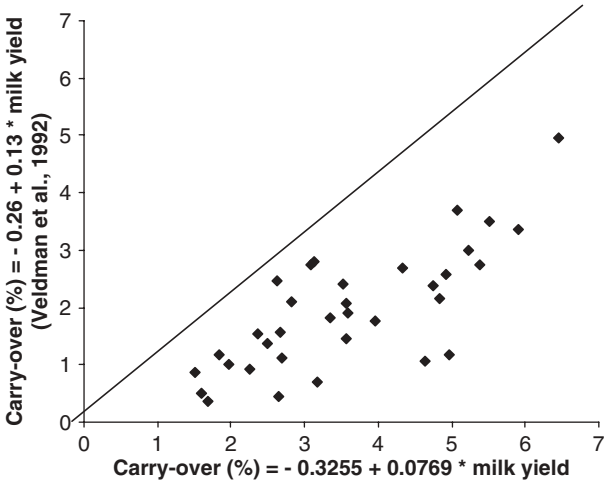

Figure 4 Plot of the predicted carry-over according to the obtained equation *v.* the predicted carry over as proposed by Veldman *et al.* (1992).

model attempts to better define the kinetics of CO of AFB1 in feed to AFM1 in milk. However, the application of the model to our data did not fit: while daily intake of AFB1 was similar among different milk-yielding groups (LY, HY), the calculated AFM1 concentrations were 1.3- and 0.9-fold the corresponding observed mean levels, respectively, for the LY and HY groups.

Mastitis as measured by high SCC could cause disruption of the tight junction of alveolar cell membranes in the mammary gland. Because of this reduction in the integrity of the blood-udder barrier, an influx of pro-inflammatory factors might further disrupt the tight junction and increased blood-udder permeability (Davis *et al.*, 1999). Similar results were observed in sheep where distended udders related to the pro-inflammatory factors have been found in the milk of sheep under similar circumstances (Colditz, 1988). Furthermore, anti-inflammatory factors from hyper-immunised cows reduced the cell membrane tight junction permeability (Stelwagen *et al.*, 1997).

The animal arrangement for the SCC content in our trial obtained groups (LY-LSCC, HY-LSCC) with average SCC below or slightly over 100 000/ml, which is considered a threshold value for a healthy udder (Walstra and Jenness, 1984; Steiner *et al.*, 1990) and groups considerably higher in SCC (LY-HSCC, HY-HSCC) in which the integrity of tight junction was probably damaged allowing leaking of blood and milk components (Bruckmaier *et al.*, 2004).

Thus, factors affecting the permeability of the blood–udder barrier, together with the low AFB1 molecular weight (312.27 formula weight), could regulate the excretion of AFM1 into milk, particularly in high-milk-producing dairy cows. From our data the increased mammary gland permeability as a consequence of inflammatory processes alone does not seem to explain the increase of the CO (Table 2).

As previously reported (Frobish *et al.*, 1986; Diaz *et al.*, 2004), the AFM1 clearance at the end of the AFB1 ingestion period was fast, bringing the AFM1 below the legislative limit (50 ng/l) within 24 h (all groups) and lower than 15 ng/l (low somatic cells groups) within 48 h from the last day of ingestion.

The AFB1 ingestion period did induce changes in some of the evaluated plasma parameters (Table 3). Even though the change in the gamma-glutamyl transferase might suggest a damage of the liver at the cellular level, the values for cows in this study were within the upper limit for cows at their stage of lactation (Bertoni *et al.*, 2000).

Conclusions

The current data suggest that milk yield is the major factor affecting the total excretion of AFM1. In this study, the CO calculated from a predictive equation was lower than previously reported for similar levels of AFB1 intake, although not outside the range. Previously reported differences in CO associated with membrane permeability due to inflammatory factors were only detectable during the first days of AFB1 ingestion and only occurred in the high-yielding cows on experiment. It is possible that the low molecular weight of aflatoxins could account for the absence of the SCC effect at plateau conditions.

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