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Re-evaluation of aflatoxin M_1 transfer into milk of high-yielding cows considering ration composition

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ABSTRACT. Aflatoxin M_1 (AFM_1) is a metabolite of aflatoxin B_1 (AFB_1) and can be detected in milk when AFB_1- contaminated feed is used. The European Commission (EC) has set maximum levels for AFM_1 at 0.050 µg/kg in milk and 0.025 µg/kg in infant formula and follow-on milk. Moreover, a maximum residue limit of 5 µg/kg AFB_1 in compound feed for dairy cattle has been established in the European Union, assuming transfer rates into milk of 2–3%. However, it has been published that transfer rates of approx. 6% may occur for high yielding cows (> 30 kg milk/day). A higher proportion of concentrates in the ration may ultimately result in a lower rumen pH or a shift in the rumen microbiome, thereby leading to changes in the rate of AFB_1 absorption. Therefore, re-evaluation of the AFM_1 transfer rate is of major importance to determine the acceptable AFB_1 intake levels from feed. The present study analysed the influence of feed composition on the transfer rate of AFB_1 to AFM_1 into milk. Cows were fed a daily low (7.5 kg) or high (12.5 kg) concentrate ration, and AFB_1 (50 µg/day) was administered orally once daily for 10 days. AFM_1 transfer rates ranged from 1.28 to 3.89%, but were not significantly influenced by the ration. Moreover, the addition of the aflatoxin binder Admonil between days 8 and 10 led to a strong reduction in AFM_1 concentrations in the milk of both groups. Based on the confirmed mean transmission rates of 2.3–2.5%, it can be concluded that the maximum limits for AFM_1 in milk (0.050 µg AFM_1/kg) according to Regulation (EC) 165/2010 will not be exceeded unless AFB_1 is introduced in feed in addition to the regulated concentrates.

Introduction

Food contaminants, such as aflatoxins, are substances that have not been intentionally added, but may be present in food as a result of contamination at various stages of its production. They can also result from environmental contamination. Not only food, but also feed may contain contaminants, for example, up to 204 µg/kg aflatoxin B_1 (AFB_1) was detected in a batch of 45 000 t of maize from Serbia which was already on the market, by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) in Germany in 2013. These concentrations clearly exceeded the permissible maximum levels of 20 µg/kg in compound feed for cattle in the European Union. Furthermore, the maximum AFB_1 content in compound feed for dairy cattle is 5 µg/kg based on a dry matter content of 88% (European Commission Regulation, 574/2011),

while the maximum level of aflatoxin M_1 (AFM_1) in raw milk, heat-treated milk and milk for the manufacture of milk-based products is 0.050 µg/kg, and 0.025 µg/kg for infant milk andfollow-on milk (European Commission, 165/2010). The German Contaminants Regulation (KmV, 2010) has further reduced the maximum level of AFM_1 for some dietary foods for babies or infants to 0.010 µg/kg. As a consequence of the incidence mentioned above, increased AFM_1 levels were detected in milk samples collected from dairy cattle fed with the contaminated maize. Moreover, approximately 1 000 farms had to suspend milk deliveries, and some had to discard milk until AFM_1 levels fell below the allowed maximum concentrations (LAVES, 2013).

Up to 6% of ingested AFB_1 can be transferred to AFM_1 in milk after metabolism in the liver of farm animals (Creppy, 2002). However, the transfer rate varies considerably among various animal species, depending on the season (Bognanno et al., 2006) and can even fluctuate between two successive milkings (Pittet, 1998). Nevertheless, it is assumed that there is a largely linear correlation between the intake of feed contaminated with AFB_1 and AFM_1 concentration in cow's milk (Fink-Gremmels, 2008). The carcinogenic potency of AFM_1 is approximately ten times lower than that of AFB_1, but based on the available data, all aflatoxins (including AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1) were classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC, 2012).

Since it is well documented that regular (long-term) consumption of food contaminated with low amounts of aflatoxins may affect human health (IARC, 2012), all efforts should be focused on reducing aflatoxin contamination of food. In line with this consideration, exposure of dairy cattle to feed contaminated with aflatoxins must be minimized in order to decrease AFM_1 levels in cow's milk as much as possible (Walte et al., 2016).

In the past, it has been reported that the transfer rate of aflatoxins in low-yielding dairy cows (< 30 kg milk/day) milked twice daily was about 1–2%, while in high-yielding dairy cows (> 30 kg milk/day), it was up to about 6% (Masoero et al., 2007; Fink-Gremmels, 2008; Britzi et al., 2013). A possible explanation for the strongly increased aflatoxin transfer rate with milk yield could be that high-yielding dairy cows are fed grain-rich diets. This in turn leads to a decrease in rumen pH (Kim et al., 2018), which can cause subacute ruminal acidosis (SARA) (Kleen and Cannizzo, 2012). The risk of developing SARA increases with a higher proportion of concentrates in the feed (Mensching et al., 2021). On the one hand, SARA may impair the barrier function of the gastrointestinal epithelium and result in a higher absorption of AFB_1 (Pantaya et al., 2016). On the other hand, rumen metabolism of AFB_1 may be altered due to SARA-associated changes in the composition of the rumen microbiota (Faniyi et al., 2019).

In Europe, low levels of AFM_1 contamination have been reported in milk, and only 0.06% of the analysed samples exceeded the European limit of 0.05 µg/kg milk (EFSA CONTAM Panel, 2004). Nevertheless, contamination of certain batches of feed can lead to widespread AFM_1 milk contamination, which should be taken into account and adequately controlled (van Asselt et al., 2017). In a long-term research project carried out in collaboration with the Milk Producer Association Schleswig-Holstein (Milcherzeugergervereinigung Schleswig-Holstein e.V., Rendsburg, Germany), AFM_1 levels in bulk milk from road tankers from the northern part of Germany were analysed between 2010 and 2017. AFM_1 levels in 95% of the analysed milk samples (n = 33 550) were below the limit of quantification (LOQ) (0.005 µg/kg) of the used method and only 0.1% of the milk samples showed AFM_1 levels ≥ 0.010 µg/kg. Similar values were obtained by Blüthgen and Ubben (2000) in the same region a decade earlier. Bulk milk samples of high-yielding dairy cows (> 30 kg milk yield/day), measured by LAVES in 2013, contained up to 0.120 µg AFM_1/kg (LAVES, 2013); based on this fact, it was deduced that the aflatoxin transfer rate was significantly higher than the assumed 1–3%, i.e. the basis for meeting the legal requirements set by the European Commission (2010). In other words, if the aflatoxin transfer rate was about 6%, then the maximum permissible AFM_1 level in milk (0.050 µg/kg) would be exceeded even if the AFB_1 concentration in the feed was not higher than the allowable 5 µg/kg of AFB_1.

In order to further reduce the uptake of AFB_1 from feed by cows, the so-called binders, based on bentonite, activated carbon or other materials, have been developed (Diaz et al., 2004). These substances are mixed with the feed, and when ingested, prevent AFB_1 absorption from the gastrointestinal tract of animals. However, the efficiency varies for different types of feed and the type and amount of binder (Giovati et al., 2015).

Considering the above, a transfer study was conducted with the following objectives: 1) to determine whether the amount of concentrate in the feed influences the aflatoxin transfer rate and whether a high proportion of concentrate in the feed may

lead to an aflatoxin transfer rate higher than 2–3%; 2) to evaluate the effectiveness of the aflatoxin binder Admonil (bentonite-montmorillonite-based; Denkavit Internationaal, Voorthuizen, The Netherlands) in reducing the aflatoxin content in the milk of cows exposed to AFB_1.

Material and methods

Animals and treatments

In order to determine the effect of ration composition on the rate of AFM_1 transfer into milk, a feeding experiment was performed at the Schaedtbek experimental station of the Max Rubner-Institute using 10 German Holstein, black and white, high-yielding dairy cows, with a herd productivity of 10 800 kg/year. The animal experiment was approved by the Ethics Committee of the Ministry of Energy, Agriculture, the Environment and Rural Areas of Schleswig-Holstein, Germany (reference number V244-3429/2016; 51-4/16), in accordance with the German Animal Welfare Act (06/2014) and Directive 2010/63/EU on the protection of animals used for scientific purposes.

The rations differed in the proportion of concentrates as the sum of concentrate feed and cereals (Table 1) and were fed from the 14th day before the start of the experiment to allow habituation. The feed components available at the experimental station were used to generate rations with a similar total composition, but maximum variation in the proportion of concentrates as the sum of concentrate feed and cereals (Table 1). The low concentrate (LC) group received 7.5 kg concentrate with a total amount of 22.5 kg dry matter per day, and the high-concentrate (HC) group was fed 12.5 kg concentrate with a total amount of 21.9 kg dry matter per day. The forage to concentrate ratios were 70:30 (LC) and 50:50 (HC) on dry matter basis. Roughage was supplied *ad libitum* as a mixture to both groups once daily. Concentrates were supplied via a computer-controlled feeding station. Diets were calculated for cows with a body weight of 650 kg and a daily milk yield of 35 kg with 4% fat. Animals had access to clean and fresh water 24 h per day.

Cows were milked in a tandem milking parlour (GEA Farm Technologies, Bönen, Germany) twice daily at 7 am and 5 pm, resulting in milking intervals of 10 and 14 h. Milk was collected into a separate milking bucket for each cow. Three main parameters were used to detect any health abnormalities: milk yield was recorded at every milking, concentrate intake was checked daily based on leftovers as a percentage of the allotted amount, and cow behaviour as monitored daily.

Table 1. Components of rations containing different proportions of concentrates (% DM)

Item	LC group	HC group
Ingredient, % of DM		
grass silage, 1st cut	10.2	12.2
grass silage, 2nd cut	12.8	15.3
grass silage, 4th cut	13.2	0
maize silage	32.1	11.5
hay	–	9.8
straw (barley)	1.1	–
concentrate feed[1]	13.7	40.1
cereals mixture[2]	15.6	10.0
minerals, salt, bicarbonate[3]	1.2	1.0
Chemical composition		
OM, % of DM	93.1	93.1
CP, % of DM	15.3	16.9
nXP, % of DM	15.3	16.3
XL, % of DM	3.4	2.3
starch, % of DM	13.5	6.3
sugar, % of DM	5.5	4.8
crude fibre, % of DM	16.6	13.1
sFibre, % of DM	11.8	10.2
ME, MJ/kg DM	9.84	10.08
NEL, MJ/kg DM	6.69	6.60

LC – low concentrates, HC – high concentrates, DM – dry matter, OM – organic matter, CP – crude protein, nXP – utilisable protein in the duodenum, XL – crude fat, sFibre – structural crude fibre, ME – metabolisable energy, NEL – net energy for lactation; [1] HaGe Primo 22/4, 22% crude protein content, energy level 4, 7 MJ NEL; [2] 20% lupin seeds, 20% wheat grains, 60% rapeseed expeller; [3] 20% salt, 20% bicarbonate, 60% minerals

To ensure intake of the intended AFB_1 dose, a gelatine capsule (length 66 mm, diameter 22 mm, volume 45 ml; Science Services GmbH, Munich, Germany) containing 50 µg AFB_1 (Aflastandard P22, R-Biopharm, Glasgow, UK) was administered orally to each cow after morning milking for 10 days; for capsule preparation, AFB_1 was diluted in acetonitrile (gradient grade; Carl Roth, Karlsruhe, Germany), the solution was then added to 12 g of ground dairy concentrate feed as adsorbent, and the capsules were firmly sealed using a 1% citric acid solution. Gelatine capsules dissolved rapidly (within minutes) in the rumen fluid, thus, AFB_1 release occurred quickly and was comparable to its intake with the feed. At days 8–10 of AFB_1 administration, the ration was supplemented with the aflatoxin binder Admonil (bentonite-montmorillonite; Denkavit Internationaal, Voort-huizen, The Netherlands), which was mixed into the rations at a dose of 150 g per cow per day, as specified by the manufacturer.

Milk and feed component sampling

Milk samples were collected from each cow at both milking times throughout the experiment, beginning 2 days before the start of supplementation (anamnesis), for 10 days of supplementation, and then followed by a 4-day phase without AFB_1 administration (wash-out period). All samples were analysed for aflatoxin M_1. After milking and thorough mixing of the composite milk, 100 ml of milk per cow were stored at 4 °C until analysis, and the remaining samples at −20 °C. The composition of the morning and evening milk of each cow was analysed twice during the experimental period by the LKV-Schleswig Holstein (Dairy Herd Improvement [DHI] samples) using infrared spectroscopy (protein, fat, lactose content) and flow cytometry (somatic cell count). Samples of all ration components, as well as the roughage mixture of both rations were tested by high-performance liquid chromatography with a photodiode array detector for AFB_1 (BVL, 2014) and no background contamination was detected.

AFM_1 quantification in milk

All morning and evening milking samples were analysed separately for AFM_1 using an in-house developed, competitive enzyme-linked immunosorbent assay (ELISA) method (Blüthgen and Ubben, 2000), which was validated in inter-laboratory tests. The limit of detection (LOD) was 0.003 µg/kg milk and the LOQ was 0.005 µg/kg with an uncertainty of 0.006 µg/kg at 0.010 µg/kg (k = 2).

Transfer rate calculation

The transfer rate was determined for days 2–7, when AFM_1 concentration in milk reached the steady state in our feeding experiment. The transfer rate was calculated as the percentage of administered AFB_1 (50 µg/cow/day) transferred as AFM_1 into milk, weighted by the milk yield from the morning and evening milking.

Statistical analyses

Statistical analysis of the transfer rate data was carried out using the statistical analysis package SAS (Version 9.4) and the implemented general linear model procedure, for which additive constants were included in the overall mean, taking into account the regression relationship to the covariate, resulting in the calculated least squares means with standard error of the means (LSM ± SEM). In this procedure, the Tukey test for multiple comparison of means was applied. Depending on the model, the factors "ration (HC/LC)", "cow within ration" and "day of supplementation" were included as additive constants and "milk yield" as a covariate. The factors parity, days in milk (DIM) and body weight showed no significant effects in the model and were therefore not included. A probability of $P < 0.05$ was used to determine statistical significance.

Results

Cows were allocated to two groups of 5 cows each to achieve a similar distribution of parity, DIM, milk yield and body weight (Table 2).

Table 2. Age, parity, days in milk (DIM), milk yield and body weight of each cow in the LC and HC group before the feeding experiment

Cow no.	Age, months	Parity	DIM	Milk yield, kg/day	Body weight, kg
LC group					
3263	72	4	215	34.1	751
3383	44	2	250	33.3	668
3429	32	1	218	33.6	613
3438	30	1	172	36.3	645
3447	28	1	101	37.7	641
Mean	41	1.8	191	35.0	664
SD	18	1.2	51	1.7	53
HC group					
3360	50	2	317	37.0	688
3372	47	2	286	35.0	655
3432	32	1	230	34.0	677
3443	29	1	161	37.7	617
3446	28	1	130	34.6	642
Mean	34	1.4	225	35.7	656
SD	9	0.5	71	1.4	28

LC – low concentrates, HC – high concentrates, SD – standard deviation

There were no statistically significant differences regarding the four aforementioned parameters between the two groups before the start of the AFB_1 administration experiment. Moreover, as shown in Table 3, there were no statistically significant differences between both groups in milk yield, milk composition and somatic cell counts on day 7 of AFB_1 administration.

Table 3. Milk yield, milk composition and somatic cell count in the LC and HC group on day 7 of AFB_1 administration

Parameter	LC group	HC group
Milk yield, kg/day	31.8 ± 2.4	33.2 ± 2.0
Protein, %	3.51 ± 0.18	3.57 ± 0.42
Fat, %	3.79 ± 0.28	4.13 ± 0.37
Lactose, %	4.79 ± 0.06	4.77 ± 0.08
SCC (in 1000/ml)	54 ± 54	108 ± 80

AFB_1 – aflatoxin B_1, LC – low concentrates, HC – high concentrates, SCC – somatic cell count; data are presented as mean value ± SD; $P > 0.05$

AFM_1 concentration in the morning milk was approximately 50% lower compared to the evening milk, which was due to the fact that the daily AFB_1 bolus was administered in the morning after milking. Therefore, AFM_1 levels in the milk samples considered for further evaluation in this report are the mean daily values of AFM_1 concentrations measured in the morning and evening milk samples (Figure 1).

difference in AFM_1 levels between the two groups was not statistically significant. The calculated mean AFM_1 concentration in milk during anamnesis was < LOD, and < LOQ during the clearance period (days 11–14) for both groups.

Figure 1 shows that the addition of the AFB_1 binder Admonil to the feed on days 8–10 of AFB_1 administration led to a strong reduction in AFM_1 concentration in milk of the cows of both groups (– 30%). Milk

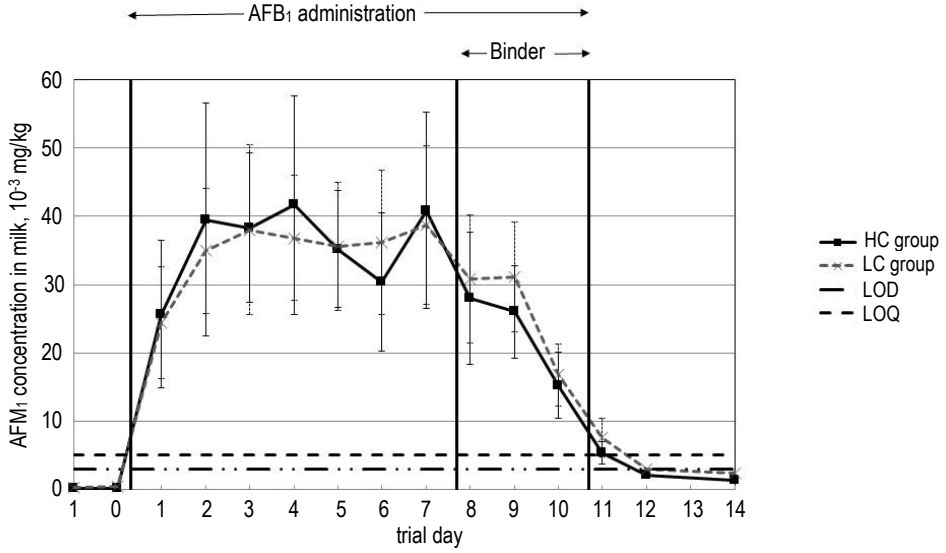

Figure 1. Process diagram of different solid-state fermentation methods with microbiological-enzymatical synergism: one-step fermentation (A) with stable parameters through the whole process and two-step fermentation (B) with changing process parameters. The dotted lines in different colours represent different process parameters.

The concentration of AFM_1 in the milk samples between days 2 and 7 of AFB_1 administration, the period when steady AFM_1 levels were observed in milk, for each individual cow, as well as the mean AFM_1 concentrations in milk of the LC and HC group are shown in Table 4. The mean AFM_1 concentration in the milk was numerically higher in the HC group than in the LC group, but the

AFM_1 content at the end of the AFB_1 administration period reached the LOQ for AFM_1 in milk.

The AFM_1 transfer rates during the steady state period for each cow, as well as the mean AFM_1 transfer rates into the milk of the LC and HC group are shown in Table 5.

The mean AFM_1 transfer rate to milk was numerically higher in the HC group (2.51 ± 0.06%)

Table 4. AFM_1 levels in milk of cows from the LC and HC groups between days 2 and 7 of AFB_1 administration (10^{-3} µg/kg)

Day	LC group					HC group				
	cow 3263	cow 3383	cow 3429	cow 3438	cow 3447	cow 3360	cow 3372	cow 3432	cow 3443	cow 3446
2	19.66	35.98	44.24	35.90	38.89	32.15	69.86	31.38	34.58	29.66
3	18.82	41.36	44.60	33.93	51.37	30.05	57.56	35.66	35.66	32.81
4	20.85	43.03	42.46	39.68	38.10	35.05	66.78	24.27	46.02	36.25
5	20.88	35.84	46.13	34.35	40.47	26.27	46.83	28.16	40.30	34.37
6	20.95	47.34	44.49	36.07	32.14	27.95	47.88	23.75	29.13	23.09
7	21.20	50.43	46.25	33.72	41.88	31.99	65.60	34.98	41.04	30.74
LSM± SEM	19.04^{e} ± 2.03	41.00^{bc}± 2.02	43.41^{b} ± 2.02	36.91^{bcd} ± 2.02	38.41^{bc} ± 2.16	32.62^{cd} ± 2.16	59.59^{a} ± 1.93	28.88^{d} ± 1.96	41.24^{bc} ± 2.54	30.70^{cd} ± 1.93
			5.76^{a} ± 0.97					38.61^{a} ± 0.97		

AFM_1 – aflatoxin M_1, AFB_1 – aflatoxin B_1, LC – low concentrates, HC – high concentrates, LSM – least square means; SEM – standard error of the mean; $^{a–e}$ – means within a row with different superscripts are significantly different at $P < 0.05$, significance test for individual cows within the group

Table 5. AFM_1 transfer rates of into milk of cows from the LC and HC groups between days 2 and 7 of AFB_1 administration, %

Day	LC group					HC group				
	cow 3263	cow 3383	cow 3429	cow 3438	cow 3447	cow 3360	cow 3372	cow 3432	cow 3443	cow 3446
2	1.22	2.01	2.65	2.33	2.45	2.04	4.23	1.95	2.35	1.87
3	1.25	2.83	2.85	2.22	3.23	2.13	3.78	2.30	2.60	2.20
4	1.31	2.71	2.68	2.83	2.55	2.47	4.43	1.66	3.22	2.30
5	1.32	2.33	3.09	2.29	2.56	1.93	3.33	1.85	2.82	2.20
6	1.28	2.89	2.71	2.36	1.87	1.87	3.11	1.46	2.10	1.49
7	1.34	3..27	2.94	2.37	2.38	2.11	4.43	2.14	2.96	2.00
LSM ± SEM	1.28^{e} ± 0.13	2.67^{bc} ± 0.13	2.82^{b} ± 0.13	2.41^{bcd} ± 0.13	2.50^{bc} ± 0.14	2.10^{cd} ± 0.14	3.89^{a} ± 0.12	1.89^{d} ± 0.13	2.69^{bc} ± 0.16	2.01^{cd} ± 0.12
			2.33^{a} ± 0.06					2.51^{a} ± 0.06		
					2.42 ± 0.73%					

AFM_1 – aflatoxin M1, AFB_1 – aflatoxin B1, LC – low concentrates, HC – high concentrates, LSM – least square means, SEM – standard error of the mean; a–e – means within a row with different superscripts are significantly different at $P < 0.05$, significance test for individual cows within the group

than in the LC group (2.33 ± 0.06%), but the difference in the AFM_1 transfer rates between the two groups was not statistically significant, and the mean transfer rate for all 10 animals (i.e. two groups combined) was 2.42 ± 0.73% (Table 5). It should be noted that the mean transfer rates calculated for individual cows strongly varied, ranging from 1.28 ± 0.13% to 3.89 ± 0.12% (Table 5).

Discussion

In the present study, AFB_1 was administered as a bolus once daily, and one might argue that this experimental design depicted an unrealistic "worst case scenario". In practice, the intake of contaminated feed by cows may be more or less evenly distributed throughout the day. However, it is also well known that *Aspergillus* species that contaminate feeds can grow in nests, leading to AFB_1 hot-spots (Vandicke et al., 2021). Consumption of feed containing such *Aspergillus* nests might in turn lead to high AFB_1 uptake in a short period of time during the day and peak AFM_1 concentrations in individual milk samples. Thus, the experimental design chosen for this study represented one of the realistic scenarios where cows could be contaminated with AFB_1 through feed. Aflatoxin levels were found to be almost negligible in the samples of finished feed tested in 2009–2011 in Central Europe, whereas in North and South America, average amounts of 7 and 2 µg/kg were measured, respectively (Rodrigues and Naehrer, 2012). However, the variance between the individual samples analysed was high as in South America up to 83 µg/kg AFB_1 and in southern Europe even 103 µg/kg were detected. Consequently, the amount of AFB_1 in less than one kilogram of such contaminated finished feed would exceed the toxin content applied in the bolus capsule in the present study.

According to Masoero et al. (2007), milk yield is a major factor affecting the total excretion of AFM_1. In the current study, the difference in milk yield between the cow with the lowest and the cow with the highest calculated transfer rate was less than 5%. Based on the fact that milk yield did not vary strongly between individual animals, our data do not support the suggestion that milk yield is an important factor for AFM_1 transfer, at least in cows producing approximately 30–35 kg milk/day. Moreover, Britzi et al. (2013) reported that the AFM_1 transfer rates of six cows ranged from 3.0 ± 0.9% up to 11.9 ± 3.3% in the mid-lactation phase (8 to 20 weeks after calving) and from 1.6 ± 0.5% to 3.5 ± 0.8% in the late-lactation phase (> 33 weeks after calving). In the present study, the cow showing the highest AFM_1 transfer rate of almost 4.0% was in the late lactation phase (cow 3372; 41 weeks after calving), while all other cows except one were in an earlier lactation phase (14 to 35 weeks after calving); one animal with the lowest AFM_1 transfer rate of about 1.3 (cow 3263) was at week 30 after calving. Thus, a correlation between the AFM_1 transfer rate and lactation status cannot be derived from the present data.

Although not statistically significant, the mean AFM_1 transfer rate into milk was numerically higher in the HC group than in the LC group. This tendency was consistent with a previous report showing that high-starch diets increased AFB_1 bioavailability, most probably by lowering ruminal pH (Pantaya et al., 2016). It should be pointed out that, as shown in previous studies as well as in the present one, AFM_1 transfer rates differ between animals. The reason for these inter-individual differences remains unclear, but the composition of the rumen microbiota, as well as acidosis, age and lactation stage of the cow

may play a role, and this issue should be analysed in future studies.

In the present study, the addition of the aflatoxin binder Admonil resulted in a significant decrease in AFM_1 concentrations in milk. This observation was in line with the results obtained by several other research groups, e.g. Kissell et al. (2013), who reported a AFM_1 level reduction up to 60% in milk after administration of bentonite to cows fed aflatoxin-contaminated diets.

Considering the calculated transfer rate of AFM_1 at 2.3–2.5% and the current AFB_1 contamination of the basic feed, it can be assumed that the maximum amount of 0.050 µg/kg in milk, as specified in Regulation of European Commission 165/2010, is not currently exceeded. In line with this statement, van Eijkeren et al. (2006) concluded in a previous study "that the European Union (EU) limit for AFB_1 in concentrate (5 µg AFB_1/kg) is adequate in preventing AFM_1 accumulation in milk exceeding the EU limit of 0.05 µg AFM_1/kg". Moreover, this statement should still be valid given the actual production conditions where concentrate constitutes approx. 30% of dairy cow dry matter intake (FAO, IDF, IFCN, 2014), and assuming that AFB_1 uptake with well-preserved maize silage in Europe is low to negligible (EFSA CONTAM Panel, 2004). However, it should be emphasized that AFB_1 levels exceeding the EU regulation for AFB_1 have been detected in the past in some maize silages in Europe (Glamočić et al., 2019). If cows consumed contaminated silage in addition to contaminated concentrate feed, both commodities containing AFB_1 content close to the permitted limit, the resulting daily intake per cow would exceed the amount of 50 µg AFB_1 to which the animals were exposed in this study. Consequently, in such a hypothetical worst case, AFM_1 milk burden exceeding the legal norm of 0.050 µg/kg for food should be expected.

Regarding AFM_1 levels in milk, particular attention should be paid to infants and toddlers, two population groups that are characterized by a relatively high milk consumption compared to their body weights. In this context, a study performed in Italy between 2013 and 2018 showed that infants and toddlers were the two most exposed population groups to AFM_1 (Serraino et al., 2019). In line with this observation, the latest Scientific Opinion of the EFSA CONTAM Panel (2020) on the risk assessment of aflatoxins in food showed that food categories "liquid milk" and "fermented milk products" were the main contributors to the overall AFM_1 mean exposure across all analysed age groups. The Panel concluded that there is a health concern, particularly for the younger age groups, based on the calculated margin of exposure values that were below 10 000 for AFM_1. In order to protect these most vulnerable group in the population, particular care should still be taken to minimize AFM_1 concentration in milk to the greatest possible extent.

Even if AFM_1 levels were high in individual milk samples, it should be considered that milk of many individual cows is pooled before leaving the farm and subsequently at the dairy, thus these steps lead to a strong dilution of AFM_1, and no increased AFM_1 exposure of adults via milk should be currently expected in Central Europe. Nevertheless, it should be mentioned that Battilani et al. (2016) calculated the increase in atmospheric temperature in Europe over the next 100 years using a model and reported that AFB_1 was expected to become a food safety issue in maize in Europe, especially in the +2 °C scenario. Therefore, continuous monitoring of feed contamination with AFB_1 and AFM_1 levels in milk will be required in the future as a preventive measure to protect the health of consumers. Whether the current AFM_1 maximum levels in milk, infant milk formula and follow-on milk set by the EC will need to be revised in the next two to three decades will depend on climate changes and the levels of feed contamination with AFB_1 and milk with AFM_1 will need to be monitored.

Conclusions

The data presented in this study do not confirm that higher concentrate proportions in dairy cattle ration lead to increased transfer rates of AFM_1 into milk. Considering the AFM_1 transfer rate of 2.3 to 2.5% confirmed in this study, and the present level of AFB_1 contamination of the basic feed, it can currently be assumed that the maximum level of 0.050 µg AFM_1/kg milk specified in Regulation (EC) 165/2010 is not exceeded. As soon as other feed sources containing AFB_1 in addition to concentrates are consumed by dairy cattle, the compliance can no longer be taken for granted.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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