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Opposite direction for seasonal variation of aflatoxin M_1 in bulk-tank milk and aflatoxin B_1 in rations: results from a prospective study in selected dairy farms of Qazvin province, Iran

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Abstract

In the present study, aflatoxin M_1 (AFM_1) in bulk milk (n=72) and aflatoxin B_1 (AFB_1) in concurrent rations (TMRs; n=48) and feed ingredients (n=230) were assessed in 12 dairy farms in winter and summer. Bulk milk was sampled on days 1, 15 and 30 of the study. Feeds were sampled at days 1 and 30. Aflatoxin was measured using ELISA kits (detection ranges: 1-81 $ngkg^{-1}$ for milk, 1.25-101.25 $ngkg^{-1}$ for feeds). AFM_1 was identified in all milk samples (range: 2.03 to >81 $ngkg^{-1}$; median: 70 $ngkg^{-1}$). Overall, 76% of milk samples (n=55/72) had AFM_1 levels <81 $ngkg^{-1}$ (Iranian limit:100 $ngkg^{-1}$). Contaminations >81 $ngkg^{-1}$ (n=17/72; 24%) were more frequent in winter (n=15/36 vs. 2/36). Sixty-nine percent of winter (n=25/36) and 31% of summer samples (n=11/36) had contaminations above the median. The chance of contaminations above the median was higher in winter (OR=5.33, P=0.007). All TMRs and ingredients had higher contaminations in summer. Seventy percent of summer and 30% of winter TMRs had contaminations above median (716 $ngkg^{-1}$). The chance of TMR contamination above median was higher in summer (OR=5.57, P=0.002). The lower AFM_1 levels in summer could be due to reduced hepatic AFB_1 metabolism and lower dry matter intake induced by heat stress. Grain mix (r_s=0.90; P=0.001), corn silage (r_s=0.66; P=0.001) and wet beet pulp (r_s=0.68; P=0.005) were the most prominent contaminants of TMRs. Due to the limitations of the diagnostic kit and different year-round nutritional conditions, higher or lower AFM_1 contaminations are probable. With the current nutritional practices, higher summer contamination may happen if heat stress is efficiently controlled.

Key words: Aflatoxin, Bulk milk, Dairy cows, Ration

Introduction

Aflatoxin M_1 (AFM_1), an immunosuppressive and carcinogenic toxin for humans, is secreted into milk after hepatic bio-transformation of dietary aflatoxin B_1 (AFB_1) (Gallo et al, 2015; Alvarado et al, 2017; Min et al, 2021), which found in many types of feedstuffs (Alvarado et al, 2017; Bahrami et al, 2016; Bilal et al, 2014; Kocasari et al, 2013; 2016; Zheng et al, 2013). The proposed world

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limits for AFM_1 in raw milk are 50 $ngkg^{-1}$ (as low as reasonably achievable) and 500 $ngkg^{-1}$ (regarding the carcinogenic effects of AFM_1) (CCFAC, 2001; JECFA, 2002). To achieve the strict limit of 50 $ngkg^{-1}$, the maximum allowable concentration of AFB_1 in dairy feeds and rations is 5,000 $ngkg^{-1}$ (European Commission, 2011; Kunsagi et al, 2012; Alvarado et al, 2017). Iranian standards for AFM_1 and AFB_1 are 100 $ngkg^{-1}$ and 5,000 $ngkg^{-1}$, respectively (ISIRI, 2016; 2020).

Some recent Iranian surveys showed the level of AFM_1 in raw and retail pasteurized milk ranged from less than 100 $ngkg^{-1}$ to more than 500 $ngkg^{-1}$ (Mashak et al, 2016; Tajik et al, 2016; Hamzeh Pour et al, 2020; Khaneghahi-Abyaneh, et al, 2020). Regarding the feedstuff contamination, the results vary between studies. Beheshti and Asadi (2014) detected AFB_1 in 19.2% of feed samples (n=146) at levels <5,000 $ngkg^{-1}$. Rezaei et al (2014) also reported similarly low levels in all of the examined samples (n=40). Bahrami et al. (2016) detected AFB_1 in 82.5% of dairy feeds (n=160), with 65% of corn silage and 10% of straw samples having contaminations above 5,000 $ngkg^{-1}$. Seasonal differences in AFM_1 in milk (Khaneghahi-Abyaneh et al, 2020; Mozaffari Nejad et al, 2019; De Roma et al, 2017; Mahmoudi and Norian, 2015; Bahrami et al, 2016; Heshmati and Milani, 2010; Kamkar, 2005) and AFB_1 in feeds (Bahrami et al, 2016; Dimitrieska-Stojković et al, 2016; Mahmoudi and Norian, 2015; Simas et al. 2007) have been reported with higher levels in cold seasons.

The year-round milk AFM_1 contamination will be best controlled with parallel assessment of milk and feeds. However, the works conducted in Iran, have addressed the contaminations of milk and feeds discretely. The aims of the present study, performed during cold and warm seasons in a number of large dairy operations, were to screen the probable hazard of AFM_1 contamination in bulk milk and defining the most critical dietary contaminants by examining the AFB_1 levels in the concurrent rations.

Materials and methods

Farms and samplings

The study was done in 12 dairy farms in Qazvin province, north-west of Iran, during winter and summer, 2019. Owing about 17,700 milking cows, the farms supplied about 40% of the total daily raw milk in the province (Cooperative Organization of Dairy Farms, Qazvin province; personal communication), with a daily milk average of 30.5 kg/cow.

Among the ration ingredients (Table 1), alfalfa hay and corn silage were stored yearly during summer and autumn, respectively. Grains were purchased several times per year and were mostly stored on the ground. Beet pulp was bought in dry form and was used dry or soaked. The rations were prepared as total mixed rations (TMR) in mixing wagons and were distributed two or three times per day. Feed refusals were swept-off daily. Toxin adsorbents (aluminosilicates) were used at about 0.6% of the ration dry matter. Cleaning of the stores, silos, preparing areas and mixing wagons was not observed during the study. Measuring milk AFM_1 in relation to feed AFB_1 was not a routine practice.

In each season, in a 30-day period, the 24-h bulk milk was sampled on days 1, 15 and 30 (n=36/season) (Procedure 87-44-05; IVO, 2005). No preservative was added. The samples were transferred cool to the laboratory and were kept frozen (-19°C) for about 30 days. Feeds were sampled, 30-days apart, two days before the first and the last milk sampling. The TMRs were sampled from the mangers immediately after the distribution of the morning meal (n=24/season) (Robinson and Meyer, 2010). The ration ingredients (Table 1) were sampled in the stores (n=230, both seasons) (Procedure 84-44-06; IVO, 2008). The wet samples (TMRs, corn silage,

soaked beet pulp) were transferred cool to the laboratory. Sampling, transferring and collecting the required data all were performed by one trained person and took about two months in each season. The experiments were done at the central diagnostic laboratory of the Veterinary Organization, Qazvin, Iran.

Table 1: Ration ingredients and their amounts in the ratios (kg DM) in the studied farms

	Winter		Summer	
	Weight range	Mean±SD	Weight range	Mean±SD
Alfalfa hay	1.14-4.17	2.3±0.83	1.64-4.53	2.78±0.96
Corn silage	4.35-13.15	7.12±0.48	3.45-9.13	5.29±1.34
Beet pulp (dry)	0.55-2.03	1.22±0.59	0.55-2.31	1.11±0.63
Beet pulp (wet)	1.00-2.37	1.22±0.47	0.81-1.44	1.19±0.22
Grain mix	13.15-15.69	14.63±1.05	12.67-16.02	14.56±1.22

Measurement of AFM_1 in milk and AFB_1 in feeds

The wet samples were oven dried at 50 to 70°C for 24-h. All samples were powdered in a clean mill and heated at 105°C for 24 hours in order to measure AFB_1 on a dry matter basis. The concentrations of AFM_1 and AFB_1 was determined using direct competitive ELISA kits (Shanghai Crystal Day Biotech, China). The sensitivity of the kits was <0.02 ng/mL for AFM_1 and <0.15 ng/mL for AFB_1. The inter- and intra-assay coefficient of variations of both tests were <8.0% and <15.0%, respectively. The analytical range of the AFM_1 kit was 1-81 $ngkg^{-1}$ and that of the AFB_1 kit was 1.25-101.25 $ngkg^{-1}$. For values out of the analytical range of the corresponding kit the lower and the upper limits were substituted as the lowest and the highest detected values, respectively. For some technical limitations and the declining effect of time on aflatoxin level (Kiermeier and Weiss, 1977), repeating the experiments with diluted samples (to reach the detection limits) was not possible.

Briefly, to measure AFM_1, 10ml of skimmed milk was mixed with 20ml of 70% ethanol. For AFB_1, a 5-gram aliquot of the powdered feed sample was mixed with 25ml of 70% ethanol. The mixtures prepared as such were centrifuged and 100µL of the supernatant was mixed with 400µL of the diluent solution. The corresponding standard solutions or the prepared samples were mixed with anti-AFM_1/AFB_1 conjugate antibody in the microplate wells. After warming, the plate was washed using the washing solution (Bio-Tek ELx50, Bio-Tek Instruments, USA). After adding the color solutions, warming and adding the stop solution, the absorbance was determined at 450nm (Bio-Tek ELx800, Bio-Tek Instruments, USA). It was inversely related to the concentration of aflatoxin.

Statistical analysis

The results were statistically studied using SPSS software (version 24, Chicago, Illinois, USA). Descriptive statistics were presented as means and standard deviations. The aflatoxin levels in milk and bunk TMR samples were classified into two relatively equal-sized groups based on their medians. Association of aflatoxin with season was evaluated using generalized estimating equations (GEE) with binomial distribution and logit function. Farm was considered as the experimental unit and was introduced into the model as subject effect. Season and number of samplings in each season were considered as repeated effects to account for the dependence between measurements. Separate models were constructed for AFM_1 and AFB_1 in milk and bunk TMR, respectively. The AFB_1 concentrations in TMRs and ration ingredients were

compared using linear mixed models. Farm was considered as random effect, and season and number of samples as fixed effects in the model. For ration ingredients, an additional fixed effect for type of feeds was introduced into the model followed by multiple comparisons with Bonferroni adjustment. The relationships between milk AFM_1 with AFB_1 in the TMR (calculated and bunk) and the separate ration ingredients were assessed by Spearman's *rho* correlation test. The P-value less than 0.05 was considered significant.

Results

Aflatoxin M_1 in milk

Aflatoxin M_1 was detected in all bulk milk samples (n=72; 100%) ranging from 2.03 to >81 $ngkg^{-1}$ (Table 2). Twenty-nine percent (n=21) of samples had contaminations <50 $ngkg^{-1}$, 47% (n=34) between 50-81 $ngkg^{-1}$ and 24% (n=17) above 81 $ngkg^{-1}$ (Figure 1A). Due to the prementioned technical problems, the latter category could potentially have contaminations below or above the Iranian standard (100 $ngkg^{-1}$). Contaminations >81 $ngkg^{-1}$ were more frequent in winter (42% in winter vs. 6% in summer; Figure 1B). The median AFM_1 concentration was 70 $ngkg^{-1}$ (considering 81 $ngkg^{-1}$ as the highest detected value), with 69% of winter samples (n=25/36) being above the median and a reverse result in summer (Table 2). The results of GEE for association of milk aflatoxin with season showed that the chance of contaminations above the median was 5.33 times higher in winter than in summer (OR=5.33, P=0.007).

Table 2: Milk AFM_1 level (ng/kg) in various samples of the studied farms during winter and summer, and the frequencies of the samples with various levels of contamination

	Winter				Summer			
Farms	1	2	3	Mean	1	2	3	Mean
1	5.13	74.89	4.74	28.16	79.59	67.41	27.86	58.29
2	69.40	>81.00	>81.00	>77.13	45.18	31.97	67.41	48.19
3	51.45	20.50	12.51	28.15	67.00	74.61	80.52	74.04
4	>81.00	>81.00	>81.00	>81.00	4.51	47.97	>81.00	>44.49
5	80.81	71.25	70.02	74.03	70.31	1.74	77.30	49.78
6	8.73	>81.00	>81.00	>56.91	>81.00	17.38	2.03	>33.47
7	>81.00	70.02	79.64	76.89	4.09	22.15	69.89	32.04
8	5.88	73.69	72.47	50.68	70.31	65.79	60.75	65.62
9	>81.00	>81.00	>81.00	>81.00	56.71	43.39	68.23	56.11
10	>81.00	>81.00	14.47	>58.82	25.73	69.47	79.59	58.26
11	4.98	>81.00	>81.00	>55.66	63.43	52.54	49.89	55.29
12	56.38	71.87	70.02	66.09	75.06	79.95	69.47	74.83
Frequencies below and above the median (70 $ngkg^{-1}$)								
				Sum				Sum
<70 $ngkg^{-1}$	7	1	3	11	7	10	8	25
≥70 $ngkg^{-1}$	5	11	9	25	5	2	4	11
Frequencies >81.00 $ngkg^{-1}$								
	4	6	5	15	1	0	1	2

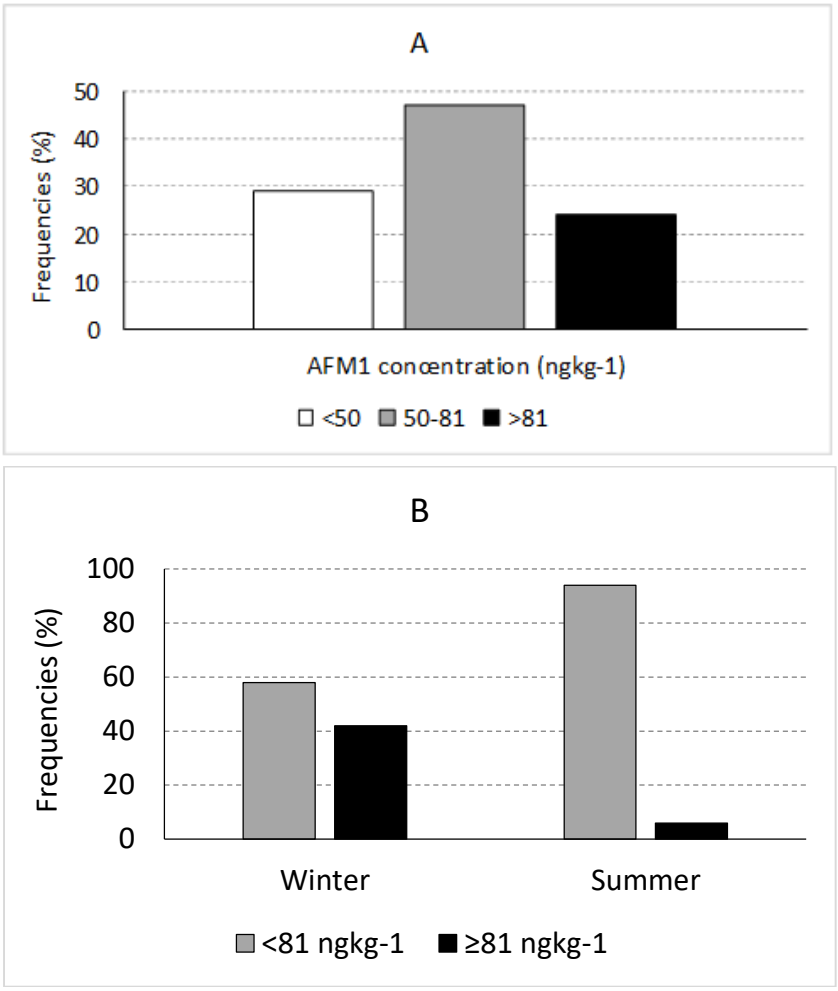

Figure 1: Frequencies (%) of bulk milk samples (n=72) with AFB_1 contaminations, A: below 50, 50-81 and above 81 $ngkg^{-1}$; B: below and above 81 ng/kg in winter and summer (n=36 per season).

Aflatoxin B_1 in Feed ingredients and TMRs

The AFB_1 contaminations of ration ingredients (n=230) are depicted in Table 3. Ten percent (n=23) of samples had non-detectable (<1.25 $ngkg^{-1}$) levels of AFB_1 and 13.9% (n=32) had values above 101.25 $ngkg^{-1}$. Contaminations were significantly higher in summer. The values >101.25 $ngkg^{-1}$ were mainly detected in summer (n=27/32) but the non-detectable values were mostly seen in winter (n=16/23).

Table 3: Aflatoxin B1 concentration (mean±SD)[1] in the ration ingredients (n=230) in the studied farms

	Number of Samples		Aflatoxin concentration (ng/kg DM)						
	Winter	Summer	Winter	Summer	Mean	Frequencies			
						<1.25		>101.25	
Feeds						W	S	W	S
Ingredients									
Alfalfa hay	24	24	39.80±38.92 [a]	65.92±43.36 [a*]	52.86±42.85 [a]	2	3	5	11
Corn silage	24	24	15.01±16.79 [b]	53.37±35.40 [b*]	34.19±33.59 [b]	4	0	0	5
BP-dry	24	23	13.94±13.19 [b]	43.11±40.49 [b*]	28.22±32.99 [b]	2	2	0	4
BP-wet	20	19	14.31±22.80 [b]	51.73±39.58 [b*]	32.03±36.68 [b]	5	1	0	3
Grain mix	24	24	15.81±21.70 [b]	43.42±33.49 [b*]	29.62±31.21 [b]	3	1	0	4
Sum	116	114				16	7	5	27

1: assuming that the values of 1.25 and 101.25$ngkg^{-1}$ (the lower and the upper limits of the experimental kit) were the lowest and the highest contamination rates; a, b: different letters indicate the significant differences in the columns (P<0.05), *: Asterix refers to significant differences in rows (P<0.05); BP: beet pulp; DM: dry matter

Table 4 shows the AFB_1 levels in TMRs on dry mater basis as "calculated" values (based on the contaminations of separate ingredients) and "bunk" values (based on the contaminations of the TMR samples taken from the bunks). The calculated values were less than the bunk values, but both were higher in summer (P<0.001). The median of bunk AFB_1 was 716 $ngkg^{-1}$ and the chance of contaminations above median was 5.57 times in summer compared with winter (OR=5.57, P=0.002). Among the bunk TMRs (n=48), the non-detectable values were seen mostly in winter (n=5/6) and all samples with AFB_1 >101.25 $ngkg^{-1}$ (n=7) were detected in summer. Seventy percent of the bunk TMR samples had contaminations above the median in summer (Figure 2).

Table 4: The concentration ($ngkg^{-1}$ DM) and the total daily content (ng) of AFB_1 (mean±SD)* in TMRs of the studied farms

TMR type	Season and sampling				Averages			
	Winter1	Winter2	Summer1	Summer2	Min	Max	Winter	Summer
Calculated								
Ration DM (kg)	25.53±2.54 (n=10)	25.04±3.00 (n=10)	23.90±2.16 (n=10)	23.31±1.66 (n=10)	20.85	31.06	25.28±2.72	23.60±1.90
							P=0.017	
AFB1 (ng/kg DM)	17.69±18.79[a] (n=10)	17.80±10.30[a] (n=10)	44.68±17.86[b] (n=10)	44.78±26.44[b] (n=10)	2.10	89.20	17.74±14.74	45.22±21.97
							P<0.001	
Daily AFB1 intake (ng)	453.06±488.08[a] (n=10)	454.72±289.94[a] (n=10)	1077.80±466.52[b] (n=10)	1067.20±607.36[b] (n=10)	48.32	2017.47	453.89±390.72	1072.50±527.12
							P<0.001	
Bunk								
Ration DM (kg)	28.43±7.04 (n=10)	25.76±6.13 (n=10)	20.90±3.43 (n=10)	21.88±3.16 (n=10)	14.17	39.45	27.09±6.57	21.39±3.25
							P=0.003	
AFB1 (ng/kg DM)	15.28±11.81[a] (n=12)	22.84±25.12[a] (n=12)	56.69±38.48[b] (n=12)	81.56±35.14[c] (n=12)	1.25	101.25	19.06±19.58	69.13±38.21
							P<0.001	
Daily AFB1 intake (ng)	496.79±438.57[a] (n=10)	577.31±680.62[a] (n=10)	1013.50±820.72[a] (n=10)	1720.06±889.36[b] (n=10)	111.63	2551.20	537.05±558.79	1375.50±905.02
							P=0.002	
Frequencies (n=48)								
<1.25 $ngkg^{-1}$	1	4	1	-		Total	5	1
>101.25n gkg^{-1}	-	-	-	7			-	7

* Assuming that the values of 1.25 and 101.25ngkg-1 (the lower and the upper limits of the experimental kit) were the lowest and the highest contamination rates; TMR: total mixed ration

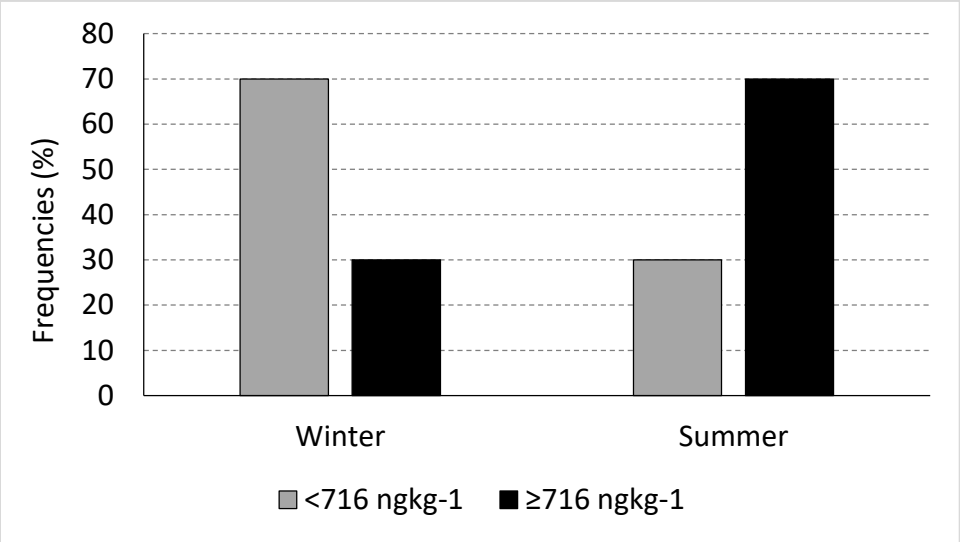

Figure 2. Frequencies (%) of bunk AFB_1 contaminations below and above median (716 $ngkg^{-1}$) during winter and summer (n=24 each season).

Correlations

No correlation was detected in neither of seasons between the ration AFB_1 and milk AFM_1. There were some correlations between the AFB_1 content of TMRs and the amount of AFB_1 added to the ration by each ingredient (Table 5). The most frequent and the strongest correlations were seen for grain mix succeeded by corn silage, wet beet pulp and dry beet pulp, respectively. The correlations were less prominent for bunk AFB_1. Calculated AFB_1 and bunk AFB_1 were related in summer (r_s=0.48, P=0.033) and in the sum of both seasons (r_s=0.43, P=0.006), but not in winter (r_s=-0.05, P=0.83).

Table 5: Correlations (r_s values) between the calculated or bunk AFB_1 levels and the aflatoxin added to the ration by each ingredient (P values in brackets)

	Calculated AFB_1			Bunk AFB_1		
	Winter	Summer	Both seasons	Winter	Summer	Both seasons
Alfalfa hay	-	-	0.31 (0.056)	-	-	-
Corn silage	0.53 (0.016)	0.52 (0.018)	0.66 (0.001)	-	-	0.31 (0.050)
Beet pulp-dry	0.61 (0.036)	-	0.42 (0.053)	-	0.67 (0.033)	-
Beet pulp-wet	-	-	0.68 (0.005)	-	-	-
Grain mix	0.82 (0.001)	0.91 (0.001)	0.90 (0.001)	-	0.45 (0.047)	0.38 (0.015)

Discussion

In the present study, AFM_1 was present in all bulk-tank milk samples (n=72; 100%) with 55 samples (76%) having levels below 81 $ngkg^{-1}$. However, the remaining samples (n=17, 24%) that had contaminations >81 $ngkg^{-1}$ (potentially above the standard limit of 100 $ngkg^{-1}$) could rise the overall contamination. Various AFM_1 contaminations of milk have been reported in other Iranian studies. Mashak et al. (2016) detected AFM_1 (15 to 140 $ngkg^{-1}$) in all (n=30) samples with 20% (n=6) above 100 $ngkg^{-1}$. Tajik et al. (2016), examining 360 milk samples, obtained an average

AFM_1 of 75.8±9.2 $ngkg^{-1}$ with 61.8% of samples having levels between 51-500 $ngkg^{-1}$ and 2.2% exceeding 500 $ngkg^{-1}$. Heshmati and Milani (2010) found AFM_1 in 55.2% (n=116/210) of samples, with 78.1% of positive samples having levels above 50 $ngkg^{-1}$ and none exceeding 500 $ngkg^{-1}$. A meta-analysis on 70 Iranian studies (Hamzeh-pour et al., 2020) showed AFM_1 contamination in 64% of raw milk samples (mean 39.7 $ngkg^{-1}$) with 75% and 9% of the positive samples having levels <50 and >500 $ngkg^{-1}$, respectively. Detection method may affect the results and less aflatoxin values are usually obtained with HPLC method compared with ELISA (Alvarado et al., 2017). So, it could be speculated in the present study that the real contamination of samples could probably be lower than the detected measures. A meta-analysis on Iranian studies (Khaneghahi-Abyaneh, et al., 2020) showed AFM_1 means of 59.19 $ngkg^{-1}$ with ELISA (55 studies; 9224 samples) and 35.23 $ngkg^{-1}$ with HPLC (18 studies; 2606 samples). Contaminations above 50 $ngkg^{-1}$ have been reported from other countries (Škrbić et al., 2014; Tsakiris et al., 2013). Škrbić et al. (2014) reported a mean of 300 $ngkg^{-1}$ AFM_1 in commercial milk samples from Serbia.

Seasonal differences in milk AFM_1 levels have been reported, pointing to higher contaminations in cold seasons (Khaneghahi-Abyaneh et al, 2020; Mozaffari Nejad et al, 2019; De Roma et al, 2017; Mahmoudi and Norian, 2015; Bahrami et al, 2016; Heshmati and Milani, 2010; Kamkar, 2005). In accordance with other studies, in the present study the contaminations above median (70 $ngkg^{-1}$) were mostly detected in winter and the chance of contaminations above median was 5.33 times in winter. Although the AFB_1 contamination of feeds has also been reported to be higher in winter (Bahrami et al, 2016; Dimitrieska-Stojković et al, 2016; Mahmoudi & Norian, 2015; Simas et al, 2007), in our study the AFB_1 levels were significantly higher in summer for all feed ingredients and TMRs. This could be due to the synergistic effects of ambient temperature and feed moisture on the behavior of mycotoxigenic fungi in summer, which is affected by climatic changes at any stage of production chain (Paterson and Lima, 2010; Magan et al, 2011; Guchi, 2015; Alvarado et al, 2017). The total daily AFB_1 contents of bunk TMRs were significantly higher in summer (Table 4). Although these levels were below the maximum allowable concentrations of AFB_1 (5,000 $ngkg^{-1}$; European Commission, 2011; ISIRI, 2020; 2016), the increasing effect of the samples with contaminations >101.25 $ngkg^{-1}$ (n=7) could not be overlooked. In addition, it is implied from some other evidences that the true contaminations of feeds could be higher. The total daily intakes of bunk AFB_1 were about 537 ng in winter and 1376 ng in summer. About 0.3 to 6.2% of AFB_1 is secreted as AFM_1 to milk (Becker-Algeri et al, 2016; JECFA, 2002) and in cows producing >30 kg milk/day, about 6% of AFB_1 may be transformed to AFM_1 (Britzi et al, 2013). In the present study, with milk production of 30.5kg/cow/day, assuming 6% bio-transformation, the total AFM_1 content of milk could range from 32 to 82.5 ng (1.05-2.7 $ngkg^{-1}$). However, many samples had contaminations >81 $ngkg^{-1}$ and the estimated AFM_1 averages were 61.25±28.91 $ngkg^{-1}$ in winter and 54.20±25.51 $ngkg^{-1}$ in summer. Thus, the true contaminations of the rations could be higher than the detected levels. These differences may partly be due to the fact that the small quantities of samples taken from huge volumes of feeds may not be representatives of all stored feeds. Increased contamination during feed processing could also have a role. The AFB_1 content of TMR samples taken from bunks was higher than that calculated from the contaminations of separate ingredients. Common devices such as mills and mixing wagons (Pinotti et al, 2016) and remainders of contaminated

feeds in wagons, preparation areas and bunks may increase the level of aflatoxin in the ration. Some of the studied farms prepared the morning rations the previous night in un-washed mixing wagons and kept it in the wagon till the next morning.

Regarding the seasonal variations in AFB_1 in feeds and AFM_1 in milk, elevated dietary AFB_1 levels in summer (OR=5.57) could have resulted in higher levels of AFM_1 in milk. However, our findings were just the opposite. A proposed reason for such findings may be the affection of liver functions by heat stress in summer (Bernabucci et al, 2010; Gallo et al, 2015; Marchese et al, 2018; Min et al, 2021). Transformation of AFB_1 to AFM_1 happens mainly in the liver by the action of cytochrome P450 enzymes (Marchese et al, 2018; Min et al, 2021), involved in a vast variety of reactions. Compromised liver functions and reduced hepatic enzyme activities have been observed in heat stressed cows (Bernabucci et al, 2010; Fan et al, 2018) and may be long lasting as it was observed in mid lactation cows (Mohebbi-Fani et al, 2020). Lower dry matter intakes in summer (Table 4) could also have a role in this finding. We conclude that the non-dietary factors affecting the metabolism of AFB_1 may fade the effect of higher intakes of toxin in summer. Paradoxically, if the general health and the dry matter intake of the cows are improved in summer (highly advised), the AFM_1 contamination of milk would potentially be elevated. Thus, the most logic way to control the aflatoxin contamination in milk is to control the critical contaminants of the rations and/or the related management insufficiencies.

The most important contaminants in the present study appeared to be grain mix and corn silage. These two, had the highest share in rations and their AFB_1 content showed the strongest correlations with TMR contaminations. Beet pulp was also an important contaminant (strong correlations) regardless of its low incorporation in rations. Alfalfa hay, which had the highest AFB_1 averages and the most frequent values >101.25 $ngkg^{-1}$ DM, showed the weakest correlations. However, it is incorporated in nearly all rations in considerable amounts in Iranian farms and may be substituted for corn silage in some conditions. In addition, the co-occurrence and the synergistic adverse effects of various mycotoxins even at relatively low levels (Kovalsky et al, 2016) should be taken into account. Mahmoudi and Norian (2015) detected the highest contaminations in corn silage followed by concentrate mix and alfalfa hay in the farms in which milk AFM_1 was assessed. Ghali et al. (2008, 2009) detected aflatoxins in 62% and 76.4% of sorghum samples using HPLC and ELISA methods, respectively. Due to the limitations of the diagnostic procedure in the present study and probably different nutritional conditions in other seasons, higher or lower overall contaminations are probable.

The general agricultural procedures for reducing mycotoxin contamination of feeds may not be applicable at dairy farms, particularly for imported feeds (mostly grains). However, some management practices at farm level may result in rapid responses. Aflatoxin M_1 enters the milk 12-24 hours after consumption and drops to non-detectable levels about 72 hours after removal of AFB_1 from ration (Pettersson, 1998). The low frequencies of milk samples with high contamination in our study could indicate relatively low contamination of the rations. Prevention of contamination of feedstuffs during processing would be an effective task. Aluminosilicates may be more efficient as toxin adsorbents than fungal cell wall which may be digested in the rumen. The lack of correlation between feed AFB_1 and milk AFM_1 could be explained by the variations in daily intake of AFB_1 and also the use of toxin adsorbents. However, without correction of some faults in the current nutritional practices, higher summer contaminations may happen if heat stress is efficiently controlled.

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Conflict of Interest

The authors declare that they have no conflict of interest in sampling, analyses and interpretation of data.

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تغییرات فصلی معکوس آفلاتوکسین M1 شیر مخزن و آفلاتوکسین B1 جیره: نتایج مطالعه‌ای آینده‌نگر در گاوداری‌های منتخب استان قزوین، ایران

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چکیده

آفلاتوکسین M_1 (AFM_1) در شیر مخزن (n=۷۲) و آفلاتوکسین B_1 (AFB_1) در جیره‌های در دست اجرا (n=۴۸؛ TMRs) و اجزای خوراک (n=۲۳۰) در ۱۲ گاوداری در زمستان و تابستان ارزیابی شدند. شیر مخزن در روزهای ۱، ۱۵ و ۳۰ مطالعه و خوراک در روزهای ۱ و ۳۰ نمونه‌برداری شد. آفلاتوکسین با استفاده از کیت‌های الایزا (محدوده تشخیص: ۱ تا ۸۱ $ngkg^{-1}$ برای شیر و ۱/۲۵ تا ۱۰۱/۲۵ $ngkg^{-1}$ برای خوراک) اندازه‌گیری شد. AFM_1 در تمام نمونه‌های شیر شناسایی شد (محدوده: ۲،۰۳ تا بیش از ۸۱ $ngkg^{-1}$ ؛ میانه: ۷۰ $ngkg^{-1}$). به طور کلی، ۷۶ درصد از نمونه‌های شیر (۵۵ نمونه از جمع ۷۶) دارای سطوح AFM1 کم‌تر از ۸۱ $ngkg^{-1}$ بودند (استاندارد ایران: ۱۰۰ $ngkg^{-1}$). آلودگی بیش از ۸۱ $ngkg^{-1}$ (۱۷ نمونه از جمع ۷۲؛ ۲۴ درصد) در زمستان شایع‌تر بود (۱۵ نمونه در مقایسه با ۲ نمونه، هر یک از جمع ۳۶). ۶۹ درصد از نمونه‌های زمستان (۲۵ از ۳۶) و ۳۱ درصد از نمونه‌های تابستان (۱۱ از ۳۶) آلودگی بالاتر از میانه داشتند. احتمال آلودگی بالاتر از میانه در زمستان بیش‌تر بود (P=۰/۰۰۷؛ OR=۵/۳۳). همه TMRها (P<۰/۰۰۲) و اجزای خوراک (P<۰/۰۰۵) در تابستان آلودگی بیش‌تری داشتند. ۷۰ درصد TMRهای تابستان و ۳۰ درصد TMRهای زمستان آلودگی بالاتر از میانه (۷۱۶ $ngkg^{-1}$) داشتند. احتمال آلودگی TMR بالاتر از میانه در تابستان بیش‌تر بود (P=۰/۰۰۲؛ OR=۵/۵۷). سطوح کم‌تر AFM_1 در تابستان می‌تواند به دلیل کاهش متابولیسم AFB_1 کبدی و مصرف کم‌تر ماده خشک ناشی از تنش گرمایی باشد. مخلوط کنسانتره (P=۰/۰۰۱؛ r_s=۰/۹)، سیلاژ ذرت (P=۰/۰۰۱؛ r_s=۰/۶۶) (r_s=0.66; P=0.001) و تفاله چغندر مرطوب (P=۰/۰۰۵؛ r_s=۰/۶۸) برجسته‌ترین آلاینده‌های TMR بودند. با توجه به محدودیت‌های کیت تشخیصی و شرایط مختلف تغذیه‌ای در طول سال، احتمال آلودگی‌های بالاتر یا پایین‌تر AFM_1 وجود دارد. در صورت تداوم روش‌های فعلی تغذیه‌ای، اگر تنش گرمایی به طور مؤثر کنترل شود، احتمال افزایش آلودگی‌های تابستانی وجود دارد.

کلمات کلیدی: آفلاتوکسین، شیر مخزن، گاو شیری، جیره

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