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Determination of Aflatoxin Levels of Feeds Used in Dairy Cow Farms and Their Effects on Blood Parameters and Milk Aflatoxin Levels in Hatay Province*

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Abstract: In the study, AFB_1 and total aflatoxin levels of roughages and concentrates from twenty dairy farms in Hatay, AFB_1 carry over rate (CO) from feed to milk as AFM_1 and correlation between total aflatoxin intake and blood parameters were investigated. In none of the roughage samples, AFB_1 levels was over 5 ppb while the concentrates of 9 farms were found to be above 5 ppb legal limit. The average AFB_1 levels of concentrates and roughages, and the average AFB_1 intake were 4.496 ppb, 1.282 ppb and, 15.987 µg/day, respectively. A total of one hundred milk samples were collected by taking five dairy cows from each farm. The two of milk samples were found to be exceeded the level of 0.05 ppb of the AFM_1 level that is the statutory limit in Turkey. The average AFM_1 level of the farms was 0.0214 ppb and none exceeded the legal limit. The average CO rate of farms was found to be 2.66%. The correlation coefficients were not significant between total aflatoxins intake and blood parameters. As a result, the AFM_1 excretion in milk was related to AFB_1 intake and milk yield. In dairy cows, a daily intake of AFB_1 37.3 µg at maximum level should not be exceeded to stay in the statutory limit for AFM_1 in milk.

Keywords: AFB_1, AFM_1, Blood Parameters, HPLC, Total Aflatoxin.

Hatay İli Süt İneği İşletmelerinde Kullanılan Yemlerin Aflatoksin Düzeylerinin Belirlenmesi ve Bu Yemlerin Kan Parametreleri ile Sütteki Aflatoksin Düzeyleri Üzerine Etkisi

Öz: Araştırmada, Hatay ilinde faaliyet gösteren 20 süt sığırı işletmesinde kullanılan kaba ve konsantre yemlerde AFB_1 ve total aflatoksin düzeyleri, yemle tüketilen AFB_1'in süte taşınma oranı (SO) ve alınan total aflatoksin düzeyleri ile kan parametreleri arasındaki korelasyon incelendi. Dokuz işletmede konsantre yem AFB_1 düzeyi 5 ppb üzerinde yer alırken, kaba yem örneklerinin hiçbirinde AFB_1 miktarı 5 ppb düzeyini aşmadı. Konsantre ve kaba yemlerdeki ortalama AFB_1 düzeyi ile ortalama AFB_1 tüketimi sırasıyla 4.496 ppb, 1.282 ppb ve 15.987 µg/gün olarak tespit edildi. Her işletmeden 5 baş süt sığırı olmak üzere toplam 100 baş hayvandan süt örnekleri alındı. İncelenen süt örneklerinden sadece ikisinde AFM_1 miktarı Türkiye için yasal limit olan 0.05 ppb üzerinde tespit edildi. İşletmelerin ortalama AFM_1 düzeyi 0.0214 ppb olarak tespit edilirken, hiçbir işletmenin ortalama AFM_1 miktarı yasal limiti aşmadı. Süte AFM_1 taşınma oranı ortalama % 2.66 olarak belirlendi. Yemle alınan total aflatoksin miktarları ile kan parametreleri arasındaki korelasyon katsayıları önemsiz bulundu. Sonuç olarak, sütle AFM_1 atılımının süt verimi ve yem tüketimi ile bağlantılı olduğu ve sütte yasal AFM_1 düzeyinin aşılmaması için sütçü ineklerde günlük 37.3 µg'ın altında AFB_1 tüketilmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler: AFB_1, AFM_1, HPLC, Kan Parametreleri, Total Aflatoksin.

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INTRODUCTION

Mycotoxins are secondary fungal metabolites synthesized by toxigenic fungi species in the presence of appropriate chemical, physical and biological factors. They can be produced in crops and food commodities during the pre- or post-harvesting period (1,2). Aflatoxins, ochratoxins, trichothecenes, zearalenones, fumonisins, tremorgenic toxins and ergot alkaloids are the mycotoxins of greatest agro-economic importance (3,4).

The maximum tolerated levels of AFB_1 in feedstuffs range from 5 to 50 µg/kg (5). The acceptable critical aflatoxin levels are 0.20 mg/kg (ppm) for single feedstuffs like corn, barley and soybeans, and 0.05 mg/kg (ppm) for mixed rations of sheep, goats and cattle (6). AFM_1 levels in milk and milk products are also quite important when human daily milk consumption is considered. In order to protect the consumer from the harmful effects of aflatoxin, the maximum level of AFM_1 in milk and milk products is restricted by many governments, as is the level of AFB_1 in feedstuffs (7). The defined maximum limit is 0.5 µg/kg in the USA and 0.05 µg/kg in the European Union, Africa, Asia and Latin America (8,9).

After biotransformation of AFB1 in the liver, it is secreted into milk in the mammary gland as AFM_1 (10). Small amounts of AFM_1 are known to appear in milk within a few hours after the feed containing AFB_1 is consumed (11). The absorption of aflatoxin and its excretion with milk as AFM_1 varies between individuals, days and lactation periods. In the literature, it was found that 3% of consumed aflatoxin in feed is transferred into milk (8,12). However, milk yield may have a large effect on the AFM_1 excretion (8,13). High-yielding dairy cows could show high excretion rates up to 6%. This is due to the relatively high consumption of concentrates and the alteration of the blood-milk barrier due to high milk production or various systemic diseases and mammary infections (9,14,15). As a rule, it is said that animals should consume less than 40 µg/d of AFB_1 in order not to exceed the allowed limits of AFM_1 in Europe (13).

Intense global commercial activity of vegetal food and raw feed materials, changing climatic conditions, increasing environmental pollution and traditional and poor agricultural practices of many developing countries could all increase the mycotoxin contamination risk in feed materials produced. In this context, it is also quite important to follow worldwide prevalence of mycotoxins for Turkey, as a food and feed importing country (16). In addition to this, it is essential for Turkey to create a local mycotoxin map to prepare a national mycotoxin strategy.

The present study was carried out to determine the presence and intensity of aflatoxin in the feed used in dairy cow farms in Hatay and in the milk of animals that consume those feeds.

MATERIALS and METHODS

For the study, a total of five Holstein cows with similar characteristics (aged from 3 to 6 years-old, weighed 350-450 kg and had passed peak lactation period) were selected from 20 different dairy farms. Every farm was considered to be one group. Procedures for management and animal care were approved by the Animal Ethics Committee of Mustafa Kemal University on 05.02.2010 (approval number; 2010/1/14).

Feed materials consisted of roughages and concentrated feeds served to animals for daily consumption. Singly collected roughage and concentrated feed samples were subjected to extraction processes according to a method as described by the AOAC (17) and analyzed with a high-performance liquid chromatography (HPLC) device (Shimadzu Class VP).

Each milk sample from the animals was taken into a 250 ml container 3-5 h after feeding. Samples were subjected to extraction processes according to the method offered by EN ISO 14501 (18) and R-Biopharm (19) and analyzed with an HPLC device

(Shimadzu Class VP). The carry over rate was calculated by using the proportion of the daily amount of consumed AFB_1 to excreted AFM_1 with daily milk production (13,14,20).

The blood samples were taken from each animal simultaneously with milk samples. After the syneresis, blood sera were analyzed to test the levels of serum triglyceride (TG), blood urea nitrogen (BUN), creatinine (CR) and total protein (TP) using the Konelab 60i Clinical Chemistry Analyzer (Thermo Electron Co, Finland) and the enzyme activities of serum aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT) using the Autoanalyzer (Refletron Roche). Total cholesterol (TC) was tested using Refletron brand kits.

Statistical Analysis

Spearman's correlation was applied to the values not showing a normal distribution after a normality test to determinate the correlation between the total aflatoxins (AFB_1, AFB_2, AFM_1, AFM_2) consumed and blood parameters, and also between the amount of AFB_1 consumed and the amount of AFM_1 formed in milk (21). All the statistical analyses were performed with the SPSS 16.0 (2007) software package.

RESULTS

A total of 68 feed samples were analyzed: 46 concentrated feed samples (67.7%) and 22 roughage samples (32.3%) collected from the farms. Detected AFB_1 and total aflatoxin levels in the feeds are given in Table 1.

None of the concentrated feed samples had an AFB_1 level exceeding 20 ppb, while samples from 9 farms (45%) had an AFB_1 level over 5 ppb. The AFB_1 level was unable to be determined in one farm's concentrates (Farm 9).

The highest AFB_1 value in concentrated feeds was 7.503 ppb (Farm 7) and the mean AFB_1 was 4.496 ppb. Commercial compound feeds had the highest AFB_1 levels among the 46 concentrated feed samples examined. The mean AFB_1 level of all samples was 3,812 ppb. Four of 15 commercial compound feeds' (27%) AFB_1 levels were determined to be above 5 ppb.

It was observed that both AFB_1 and total aflatoxin levels of roughage samples were lower than in concentrated feeds. None of the measured values of roughages exceeded an AFB_1 level of 20 ppb, as the statutory limit for feed commodities in Turkey. However, in roughage samples gathered from 8 farms (40%), no AFB_1 level could be determined. None of the 22 samples were over 5 ppb. The highest value of AFB_1 in the roughage samples was 4.711 ppb (Farm 11), and the mean was 1.282 ppb.

In the study, 2% of 100 total milk samples gathered from 20 farms were above the 0.05 ppb limit AFM_1 level (Table 2). The average AFM_1 level (0.0214 ppb) of all farms was under 0.05 ppb, as the statutory limit in Turkey and the EU. AFM_1 levels were detected to be between the 0.02-0.04 ppb range in 11 farms (55%) and under 0.02 ppb in 8 farms (40%). Only one farm's AFM_1 level reached 0.04-0.05 ppb, which is on the verge of unacceptable level of AFM_1.

Farm-based means of blood parameters analyzed including the activities of AST, ALT, AP, GGT, and the levels of creatinine, cholesterol, triglycerides, total protein and blood urea nitrogen were given in Table 3. No correlation was found between the total amounts of aflatoxin consumed with feed and blood parameters (Table 4).

Table 1. Detected AFB_1 and total aflatoxin levels of feed samples in farms.
Tablo 1. İşletmelerdeki yem örneklerinde tespit edilen AFB_1 ve toplam aflatoksin düzeyleri.

Farms	AFB_1 (ppb)				Total Aflatoxin (ppb)			
	Concentrates	/	Roughages	Total AFB_1 Values of Farms	Concentrates	/	Roughages	Total Aflatoxin Values of Farms
1	3.543	/	ND	3.543	7.151	/	ND	7.151
2	0.098	/	1.571	1.669	0.118	/	2.596	2.714
3	2.823	/	ND	2.823	2.897	/	ND	2.897
4	4.983	/	1.314	6.297	17.235	/	3.241	20.476
5	7.396	/	ND	7.396	21.640	/	ND	21.640
6	4.758	/	ND	4.758	5.512	/	ND	5.512
7	7.503	/	2.418	9.921	16.342	/	4.107	20.449
8	0.722	/	ND	0.722	1.297	/	ND	1.297
9	ND	/	3.131	3.131	ND	/	4.914	4.914
10	7.166	/	ND	7.166	18.192	/	ND	18.192
11	3.882	/	4.711	8.593	4.013	/	11.132	15.145
12	6.260	/	ND	6.260	6.581	/	ND	6.581
13	7.239	/	2.420	9.659	22.033	/	2.420	24.453
14	5.341	/	2.466	7.807	12.920	/	5.855	18.775
15	6.735	/	ND	6.735	14.750	/	ND	14.750
16	5.484	/	1.436	6.920	12.807	/	3.532	16.339
17	4.134	/	2.158	6.292	4.492	/	2.840	7.332
18	5.217	/	0.536	5.753	10.381	/	1.074	11.455
19	4.797	/	1.968	6.765	5.662	/	5.615	11.277
20	1.847	/	1.506	3.353	4.281	/	1.506	5.787
Average	4.496	/	1.282	5.778	9.415	/	2.442	11.857

ND: Not Determined (Tespit Edilmedi).

Table 2. General profile of animals presented in farms and transition rate of AFB_1 into milk as AFM_1 (carry over rate).
Tablo 2. İşletmelerde mevcut hayvanlara ait genel profil ve AFB_1'in süte AFM_1 olarak geçiş düzeyi (süte geçiş oranı).

Farm	AFB_1 of Roughages	AFB_1 of Concentrates	Total AFB_1 Intake	Milk AFM_1 Levels	Average Milk Yield	Carry Over Rate
	(µg/day)	(µg/day)	(µg/day)	(µg/l)	(kg/day)	(%)
1	0.000	6.838	6.838	0.0091	21.0	2.81
2	6.928	0.283	7.211	0.0104	19.0	2.75
3	0.000	8.006	8.006	0.0128	17.4	2.99
4	4.625	11.628	16.253	0.0214	21.0	2.77
5	0.000	29.769	29.769	0.0368	25.0	3.09
6	0.000	10.420	10.420	0.0126	23.0	2.77
7	8.777	18.182	26.959	0.0372	20.4	2.81
8	0.000	1.537	1.537	0.0018	20.8	2.41
9	17.189	0.000	17.189	0.0204	27.0	3.21
10	0.000	27.870	27.870	0.0337	27.0	3.26
11	24.627	13.509	38.136	0.0462	26.0	3.15
12	0.000	19.144	19.144	0.0234	21.0	2.57
13	5.881	11.707	17.588	0.0254	10.8	1.56
14	5.129	7.421	12.550	0.0211	12.4	2.08
15	0.000	13.317	13.317	0.0182	20.0	2.73
16	4.480	11.381	15.861	0.0231	17.0	2.48
17	5.697	7.288	12.985	0.0208	15.0	2.40
18	2.231	14.608	16.839	0.0211	23.4	2.94
19	5.156	8.452	13.608	0.0197	14.0	2.03
20	4.202	3.454	7.656	0.0108	16.8	2.37
Average	4.746	11.241	15.987	0.0214	19.9	2.66

Table 3: Farm-based means of blood parameters analyzed.
Tablo 3: Analiz edilmiş kan parametrelerinin işletme bazlı ortalamaları.

Farm	AST	ALT	ALP	GGT	TC	BUN	CR	TP	TG
	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(mg/dl)	(mg/dl)	(mg/dl)	(g/dl)	(mg/l)
1	93.76	24.48	50.60	20.04	211.00	20.20	1.12	7.30	19.20
2	166.60	40.90	64.56	37.48	272.00	17.00	0.76	5.84	16.00
3	119.80	54.00	48.26	43.42	217.60	27.60	0.94	6.28	18.20
4	129.20	50.62	44.46	23.92	277.80	11.20	0.98	6.96	21.00
5	128.32	30.90	47.76	41.66	140.20	18.40	1.04	6.60	10.60
6	101.22	32.04	34.04	51.52	136.60	15.60	0.96	6.62	18.40
7	126.46	60.24	54.10	30.92	252.00	15.40	0.98	6.28	18.20
8	128.34	47.88	63.04	37.68	239.20	8.40	1.06	6.46	14.80
9	119.80	50.62	59.34	33.04	273.60	17.80	0.84	6.88	13.80
10	115.60	49.84	58.78	33.20	215.80	19.80	0.90	6.44	15.20
11	135.32	52.76	54.84	30.20	268.80	15.40	0.90	6.80	9.40
12	117.54	35.98	63.40	27.44	240.20	14.60	0.98	6.46	7.40
13	113.94	31.72	63.06	27.72	189.80	12.80	0.98	6.46	24.80
14	105.62	31.08	64.04	42.38	162.60	16.60	0.98	6.82	7.80
15	128.40	39.28	41.44	28.62	287.80	19.20	1.04	6.86	8.80
16	96.82	40.20	62.52	23.44	193.60	12.80	0.98	6.96	15.00
17	113.78	31.72	52.40	26.44	124.00	14.60	1.14	6.84	14.00
18	106.00	20.28	66.08	32.10	205.40	16.20	1.08	6.52	6.60
19	107.58	31.44	42.24	23.56	156.60	13.40	0.94	7.14	15.80
20	127.04	33.02	40.44	26.28	211.20	10.00	1.20	7.38	7.60
Average	119.06	39.45	53.77	32.05	213.79	15.85	0.99	6.70	14.13

AST: Aspartate Aminotransferase; ALT: Alanine Amino Transferase; AP: Alkaline Phosphatase; GGT: Gamma-glutamyl Transferase; TC: Total Cholesterol; BUN: Blood Urea Nitrogen; CR: Creatinine; TP: Total Protein, TG: Triglyceride.

Table 4. Farm-based correlations between total aflatoxin intake and blood parameters.
Tablo 4. Toplam aflatoksin tüketimi ile kan parametreleri arasındaki işletme bazlı korelasyon.

	Parameters								
Total Aflatoxin Intake	AST	ALT	AP	GGT	TC	BUN	CR	TP	TG
Correlation Coefficients	0,110	0,062	0,050	-0,146	0,057	0,045	-0,109	0,010	-0,070
P	0,322	0,398	0,418	0,27	0,405	0,425	0,324	0,484	0,385
N	20	20	20	20	20	20	20	20	20

AST: Aspartate Aminotransferase; ALT: Alanine Amino Transferase; AP: Alkaline Phosphatase; GGT: Gamma-glutamyl Transferase; TC: Total Cholesterol; BUN: Blood Urea Nitrogen; CR: Creatinine; TP: Total Protein, TG: Triglyceride.

DISCUSSION and CONCLUSION

Nutritional materials with available carbohydrate and fat contents get moldy faster (22). Because concentrated feeds have more available carbohydrate and fat contents, they generally have more aflatoxin than roughages.

The findings of the present study are in parallel with the findings of similar previous studies (23-26) showing that the concentrates, primarily commercial compound feeds, had a higher proportion of aflatoxin level compared to other feed materials. In contrast with these findings, Polat (27) found higher AFB_1 rates in roughages compared to the concentrated feeds. However, the higher amount of mycotoxins in roughages could have been based on the tough climatic conditions of the area and/or warehousing feeds contaminated with rainwater or snow that were not well-dried after harvesting.

The term carry-over (CO) refers to the passage of undesired compounds from contaminated feed into food of animal origin (28). It is determined by the proportion of the daily amount of consumed AFB_1 to excreted AFM_1 with daily milk yield by many researchers (13,14,20). In a meta-analysis of transition rates, Pettersson (29) found that the CO rate of AFB_1 to milk was between 0.18-3.24% before 1985 and 0.32-6.2% after. Similarly, Polovinski-Horvatovic et al. (30) reported a CO level from feed to milk ranging from 0.3 to 6.2%. Several researchers reported that there was a linear relationship between the amount of AFM_1 in milk and AFB_1 in feed consumed by dairy cattle (31). Similarly, Karakaya and Atasever (32) reported a positive correlation (+0.329) between the AFB_1 contents of feed and the AFM_1 level of milk. Even so, it was stated that milk yield is the major factor affecting the AFM_1 excretion. Thus, higher milk yield results in higher AFM_1 excretion (8,14,15). Veldman et al. (13) reported that 40 kg/d milk yielding cows show a higher CO rate (3.8%) than lower yielding cows with 16 kg/d. Similarly, Britzi et al. (33) determined that the average CO rate was 2.5% for under 35 kg/d milk yielding cows in late lactation, while it was 5.4% for over 35 kg/d milk yielding cows in mid-lactation. In the present study, the mean milk yield of 100 cows as all passed the peak lactation period was 19.9 kg/d, and the measured carry-over rate was 2.66% (min. 1.56%, max: 3.26%). There was a linear relationship between the consumed AFB_1 and the excreted AFM_1 into milk (r^2: 0,940).

A seasonal effect of aflatoxin M_1 concentration has been reported in some studies, in which it was observed a higher concentration of AFM_1 in cold seasons than in hot seasons because farmers tend to use higher amounts of compound feeds in winter (34). In other words, animals consume less concentrated feeds in summer because they also graze on pasture. In a previous study, Çeçen (35) showed that pasturing has an important effect on the decrease of AFM_1 in milk. In that study, 30 samples from animals feeding with compound feeds in the barn and 31 samples from pasturing animals were investigated. It was found that only one milk sample of pasturing animals had AFM_1 (3.22%), while 23 samples from animals feeding in the barns (76.66%) had AFM_1. The present study was carried out in summer, so it is likely that the AFM_1 data obtained was affected by pasturing and the use of a lower amount of concentrates in the rations. It was determined that 0.0214 ppb AFM_1 was formed in the milk of cows consuming 15.987 µg/d of AFB_1. In accordance with this, it was determined that in order not to exceed the 0.05 ppb statutory limit of AFM_1 for Turkey and the EU, animals should consume below 37.3 µg/d AFB_1. These results are similar to the findings of Veldman et al. (13) who reported that daily consumption of AFB_1 levels should be less than 40 µg so as not to exceed the maximum AFM_1 limit.

In the present study, no correlation was observed between the total aflatoxin intake, the activities of AST, ALT, AP and GGT, and the levels of creatinine, cholesterol, triglycerides, total protein and blood urea nitrogen. These results are similar to those of previous studies indicating that the parameters studied were also unaffected by aflatoxin intake (36,37). However, there were different results for some of these parameters in some previous studies (24,38). The reason for these differences is likely due to the individually varying effects of aflatoxin intake on blood parameters or the intensity of the dose received, as well as whether there was chronic or acute aflatoxin exposure.

As a result, the average carry-over rate of AFB_1 in feedstuffs to AFM_1 in milk was measured as 2.66% in the present study; therefore, it is concluded that animals should consume AFB_1 below 37.3 mg/d so as not to exceed the statutory AFM_1 limit of dairy cows in Turkey.

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