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Aflatoxin, heavy metal and pesticide residue contents of some compound feeds produced in Turkey

Türkiye'de üretilen bazı karma yemlerin aflatoksin, ağır metal ve pestisid kalıntı düzeyleri

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ABSTRACT

The objective of this study was to determine the residue based pollution levels of some compound ruminant and poultry feeds produced in different regions of Turkey. A total of 100 feed samples (lactating cow, cattle fattening, lamb fattening, cage layer and broiler finishing feeds) collected according to official sampling methods at 3-month intervals from commercial feed compounders in 5 regions where animal and feed productions were most intensive were analyzed for aflatoxins (B_1, B_2, G_1, G_2, total aflatoxin), heavy metals (Pb, Cd, Hg, As) and organophosphorus and organochlorine pesticides. High performance liquid chromatography (HPLC) analysis indicated that 70 samples contained total aflatoxin (TAFL), 67 contained aflatoxin B_1, B_2 was observed in 41, G_1 in 1, and G_2 in 2 samples, while; however, none of them exceeded the maximum residue levels (MRL) of the "Instruction about Undesired Materials in Feeds" prepared by Turkish Ministry of Agricultural and Rural Affairs (TMARA). There were differences in the amount of samples obtained in January, April, July and October. Similar differences existed among the regions, and also among the feed types analyzed. No pesticide residue was found in any feed sample in gas chromatography/mass spectrometry (GC/MS) analysis, except 2.21, 2.51, 2.61, 1.88 and 2.87 ppm quintozone in lactating cow, cattle fattening, lamb fattening, broiler finishing and cage layer feeds, and 0.10 and 0.13 ppm diazinon in lamb fattening and cage layer feeds, respectively. Since, the instruction of TMARA did not contain permitted maximum residue levels (MRL) related to pesticide residues in compound feeds, it was not possible to make any comparison on the data obtained. In terms of heavy metals, mercury (Hg) and arsenic (As) levels exceeded MRL permitted in the instruction only in two, and lead (Pb) in one sample.

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ÖZ

Bu araştırmanın amacı, Türkiye'nin farklı bölgelerinde üretilen karma yemlerdeki aflatoksin (B_1, B_2,G_1, G_2, toplam aflatoksin), ağır metal (Pb, Cd, Hg, As) ve organik fosforlu ve organik klorlu pestisid kirlilik düzeylerini tespit etmektir. Bunun için, 1 yıl boyunca, 3'er aylık aralıklarla, hayvancılığın ve karma yem üretiminin en yoğun olduğu 5 bölgedeki karma yem fabrikalarından, usulüne göre alınan, toplam 100 adet süt, sığır besi, kuzu besi, yumurta kafes ve etlik piliç bitirme yem örnekleri üzerinde aflatoksin, ağır metal ve pestisid analizleri gerçekleştirilmiştir. Yüksek basınçlı sıvı kromatografisi (HPLC) kullanılarak yapılan aflatoksin analizleri sonucunda, 70 yem örneğinde toplam aflatoksin (TAFL), 67 örnekte aflatoksin $B_{1,}$ 41 tanesinde B_2, 2'sinde G_2 1'inde G_1, saptanmış; ancak, bunların hiç birisi Tarım ve Köyişleri Bakanlığı'nın Yemlerde İstenmeyen Maddeler Hakkında Tebliğ'inde izin verilen en yüksek kalıntı düzeylerini aşmamıştır. TAFL ile B_1 ve B_2 aflatoksinleri görülme sıklığı bakımından Nisan, Temmuz, Ocak ve Ekim örnekleri arasında farklılıklar görülmüş; benzer farklılıklar bölgeler ve yem çeşitleri arasında da gözlenmiştir. Gaz kromatografisi / kütle spektrometresi (GC/MS) ile yapılan pestisid analizlerinde süt, sığır besi, kuzu besi, yumurta ve etlik piliç yemlerinde, sırasıyla 2,21, 2,51, 2,61, 1,88 ve 2,87 ppm quintozone, kuzu besi ve yumurta yemlerinde 0,1 ve 0,13 ppm diazinon bulunmuş; diğer örneklerde pestisid kalıntısına rastlanmamıştır. Bakanlığın tebliğinde karma yemlerde pestisidler için kabul edilebilir en yüksek değerler (MRL) verilmediğinden, karşılaştırma olanağı bulunamamıştır. Atomik absorpsiyon spektrofotometresi (AAS) ile yapılan analizlerde, sadece 2 örnekte civa (Hg), 1 örnekte de kurşun (Pb) kalıntı miktarları tebliğde izin verilen maksimum değerleri aşmıştır.

1. Introduction

When animals consume polluted feeds, aflatoxin, heavy metal and pesticide intakes may reach up to levels threatening human health by transferring into end products such as meat, eggs and milk. In order to prevent animal foods becoming harmful to the consumers, feeds utilized should be kept under control (Kurtoğlu and Coşkun 2001).

Various aflatoxins produced by different *Aspergillus flavus* species are B_1, B_2, G_1, G_2, M_1 and M_2. Among them, the most frequently existing one in moldy feeds and processed foods is B_1; while, the most rare is B_2 (Çelik 2001). Aflatoxin B_1 (AFB1) takes place in the group designated as human carcinogens by International Agency for Research on Cancer (IARC) in 1993 (Smith 1997). Aflatoxins can easily affect the materials such as corn, wheat, cottonseed meal, soybean meal, sunflower meal, animal originated feeds and compound feeds even at normal room temperatures (Çelik 2001).

Heavy metal pollution is a serious problem resulted from industrialization in all over the world. It is proved that metals accumulate in air, soil and water. They are released into the environment from industrial plants as well as mining activities (Tunçoku and Çınar 1995). Similarly, some of the important sources of mercury (Hg) are industrial branches such as electrochemistry, paint, drug, paper and metallurgy. Other important sources of Hg are Hg containing compounds utilized as fungicide for cereals (Şanlı 1976).

Residues in feeds and foods caused by pesticides create serious problems all over the world. Since, the rate of alternative systems such as biologic and organic farming is low, pesticides are used very intensively in today's modern agriculture. Mistakes in practical application increase the danger. It has been postulated that due to the intensive production methods applied to the agriculture and the fast developing industrial affairs, problems related to aflatoxin, heavy metal and pesticide pollutions in feed materials, and compound feeds as their end products produced in the country, should be expected.

Therefore, the objective of this study is to determine the pollution levels and seasonal variations of polluting agents in some compound ruminant and poultry feeds (lactating cow, cattle fattening, lamb fattening, cage layer and broiler) produced in different regions of Turkey where feed and animal productions are the most intensive, and furthermore, to determine whether legal tolerance levels have been exceeded or not.

2. Materials and Methods

A total of 100 compound lactating cow, cattle fattening, lamb fattening, cage layer and broiler feed samples were collected at 3-month intervals during a whole year (25 samples for each period) from commercial feed factories in 5 provinces at Aegean, Marmara, Thrace, Eastern Anatolia and Central Anatolia regions of Turkey where feed and animal productions are the most intensive. Samples protected at -18ºC were analyzed for aflatoxsins (B1, B2, G1, G2 and total); heavy metals (Pb, Cd, Hg, As); organochlorine (Trifluralin, Gamma-HCH (hexachlorohexan), Quintozone, Triadimefon, Captan, Alpha Endosulfan, 4-4 DDT (dichlorodiphenyl trichloroethane), Beta Endosulfan, 2-4 DDT, Endosulfan Sulfate) and organophosphorus pesticides (Dichlorvos, Diazinon, Chlorpyrifos Methyl, Metalaxyl, Fenitrothion, Malathion, Parathion Methyl, Chlorfenapyr, Triazophos).

2.1. Aflatoxins analysis

Aflatoxin analyses were carried out by HPLC (high performance liquid chromatography) with ODS2 column and Cobra cell derivative of aflatoxins (Vicam 1999). The limits of detection (LOD) values utilized were 0.20 ppb (µg kg-1) for AFB1, 0.10 for B2, 0.51 for G1 and 0.17 for G2.

2.2. Heavy metals analysis

Lead (Pb) and cadmium (Cd) were analyzed by atomic absorption spectrophotometer's (AAS) Graphite Furnace system; arsenic (As) and mercury (Hg) with Hydrous system (Jorhem 1993; Skurikhin 1993). LODs for Pb, Cd, As and Hg were determined as 0.0003, 0.02, 0.004 and 0.000006 ppm (mg kg-1), respectively by using certified standard reference material (CRM).

2.3. Pesticides residue analysis

Following oil extraction of the samples and pesticide separation from the oil, pesticide analysis of the extracts was realized in gas mass spectrometer (GC/MS) as detailed in Pesticide Analytical Manual (PAM 1999). Quintozone recovery value (in 500 ppb) was estimated as 62.30% for lactating cow, 64.16% for cattle fattening, and 61.64% for lamb fattening feeds. Similarly, diazinon recovery value (in 50 ppb) was found to be 69.19% for lamb fattening feed.

2.4. Statistical analyses

Since no data at measurable levels were obtained from some samples for all criteria evaluated, statistical analysis was not applied to the data collected.

3. Results

Data obtained from aflatoxin analysis of compound feeds were presented in Table 1. 70 samples contained total aflatoxin (TAFL); 67 contained AFB1; B_2 existed in 41; G_2 in 2 and G_1 in 1 samples. TAFL was detected in 20 samples of 25 feeds obtained in April; however, this figure 18 in July, 17 in January, 15 in October; while the B_1 figures were 14 in January; 20 in April, 18 in July, 17 in January, and 15 in October. Similarly B_2 was observed in 13, 12, 10, and 6 samples in the same seasons, respectively. Considering AFB1 data specifically, levels in 34 samples were found to be between 0 to 1, 1 to 3 in 25 samples, 3 to 5 in 6 samples, 13.9 in one and 16.0 ppb in another.

Table 1. Numbers of feed samples containing detectable levels of various aflatoxins.

Aflatoxin	CF[1]	LC	LF	CL	BF	Max. (ppb)
B_1	10	16	11	15	15	16.0
B_2	7	8	8	8	10	1.88
G_1	0	1	0	0	0	1.67
G_2	1	0	1	0	0	0.33
TAFL[2]	11	16	12	15	16	17.88

[1]CF: Cattle fattening, LC: Lactating cow, LF: Lamb fattening, CL: Cage Layer, BF: Broiler finishing
[2]Total aflatoxin (B_1+B_2+G_1+G_2)

Table 2. Pb, Hg and As exceeding MRL[1] in feeds collected from different regions of Turkey.

	Region[2]	Season	Feed[3]	Max. (ppm)	MRL (ppm)	Number of samples exceeding MRL (ppm)
Pb	Aegean	October	LC	5.59	5	1
Hg	Eastern Anatolia	July	CF	0.16	0.1	1
	Aegean	July	LF	0.15	0.1	1
As	Eastern Anatolia	July	LC	2.45	2	1
	Eastern Anatolia	July	LF	2.98	2	1

[1]MRL: Maximum residue level
[2]Regions: Aegean, Eastern Anatolia
[3] CF: Cattle fattening, LC: Lactating cow, LF: Lamb fattening, CL: Cage Layer, BF: Broiler finishing

Results of Pb, Hg and As analysis exceeding MRL of the TMARA have been shown in Table 2. Hg and As levels exceeded permitted MRL in 2 and Pb in only 1 sample. None of Cd levels exceeded MRL values mentioned before.

No pesticide residue quintozone was detected as 1.88, 2.21, 2.51, 2.61, and 2.87 ppm in broiler finishing, lactating cow, cattle fattening, lamb fattening and cage layer feeds provided from Eastern Anatolia in January, respectively (Table 3). Similarly diazinon was found as 0.10 and 0.13 ppm in lamb fattening and cage layer feeds received from Central Anatolia in April, respectively.

Table 3. Pesticides detected in feeds collected from different regions.

Pesticide	Region[1]	Season	Feed[2]	Level (ppm)
Quintozone	Eastern Anatolia	January	BF	1.88
	,,	,,	LC	2.21
	,,	,,	CF	2.51
	,,	,,	LF	2.61
	,,	,,	CL	2.87
Diazinon	Central Anatolia	April	LF	0.10
	,,	,,	CL	0.13

[1]Regions: Eastern Anatolia, Central Anatolia
[2]CF: Cattle fattening, LC: Lactating cow, LF: Lamb fattening, CL: Cage Layer, BF: Broiler finishing

4. Discussions

MRL figures permitted have been given in the “Instruction about Undesired Materials in Feeds” (Resmi Gazete 2010) prepared by Turkish Ministry of Agricultural and Rural Affairs (TMARA) only for AFB1 (Table 4). AFB1 data obtained in the present study did not exceed MRL of the TMARA. Surai et al. (2004) investigated feed, milk and cheese samples collected from the farms and markets in Western Sicily for AFB1 and M_1. HPLC analysis indicated that AFB1 varied between 7.69-10.00 ppb; and none of the samples was over the legal limits of European Union (5 ppb).

In a study realized by Çelik and Öztürkcan (2000) on 8 different feed materials (wheat grain, cottonseed meal, corn grain, alfalfa hay, wheat bran, broiler feed, lactating cow feed and processed peanut) the ratio of AFB1 containing feeds was reported as 20.3%. However, when they compared, none of the AFB1data was over the risk levels of American Food and Drug Administration (FDA).

Table 4. Legal MRL released by TMARA[1] for feeds analyzed[2].

Undesired substances	Complete feeds (12% humidity)	MRL (ppm)
As	All	2.0
Pb	All	5.0
Hg	All	0.1
Cd	Cattles, sheeps, goats (except calves, lambs and kids)	1.0
	Others (except pets)	0.5
Aflatoxin B1	Lactating cow	0.005
	Lamb	0.01
	Poultry and pig	0.02
DDT3	All	0.05

[1]TMARA: Turkish Ministry of Agricultural and Rural Affairs
[2] Resmi Gazete (2010)
[3] DDT: Dichlorodiphenyl trichloroethane

In another study conducted by Yıldız (2003) on corn, wheat, barley, row and processed full fat soybean, sorghum, regular wheat bran, fine wheat bran, maize bran, sunflower meal (SFM), cottonseed meal (CSM), soybean meal (SBM), peanut meal (PM) and hazelnut meal (HM), 6.3% of samples consisted TAFL, 51.4% zearalenone, 42.4% ochratoxin A. The ratio of TAFL contents exceeding 20 ppb was 8.7%, zearalenone over 8 ppb was 22%, ochratoxin higher than 50 ppb was 9.3%. The figures exceeding limits were mostly obtained from corn, wheat, SBM, CSM and SFM.

According to Cespedes and Diaz (1997) AFB1 was found in 11 of 50 sorghum, 4 of 33 corn, 15 of 17 CSM, 12 of 30 poultry feed and 7 of 16 pig feed samples and only 9 of them exceeded current legal limits in Colombia.

Altuğ and Beklevik (2003) who studied on 85 compound fish feed samples collected from domestic fisheries and compounders, being some of them imported, determined total aflatoxin contents, between 21.2 to 42.4 ppb in 20 samples. Figures belonging to the samples collected from fisheries were found to be higher than both imported feeds and materials from the compounders. Since aflatoxin levels are correlated to environmental conditions as well as dry matter content of the feed, storage humidity should be below 10% for a safe deposition; when it exceeds 12.5%, feeds must be consumed immediately in the first week (Gowda et al. 2003).

In the present study, aflatoxin levels were higher in April and July samples than the other periods. This may be attributed to probable high moisture contents of the feeds.

Differences in aflatoxin levels in terms of seasons, regions and feed types are expectable since humidity and temperature may affect fungal development (Çelik et al. 1999, Çelik 2001). In other words, drying process applied to the feeds and the environmental factors such as storage temperature and humidity (Gowda et al. 2003), as well as processes such as dry or steam heating or coking extrusion and pelleting may create significant differences in aflatoxin levels. Besides, some materials in the feed mixture such as corn, wheat, oil seed meals and animal originated feeds and alfalfa hay are more susceptable to molds (Altuğ et al. 1995, Yıldız 2005). In fact, Dehuri et al. (1994) indicated that AFB1was at the highest levels during rainy seasons in broiler starter, corn and fishmeal.

According to the TMARA instruction, legal MRL values are 2 ppm for As, 5.0 for Pb, 0.1 for Hg in all species; and 1 for Cd in cattle, sheep and goat complete feeds (excluding lamb calves and kids), 0.5 for others (except pets). A total of 83 feeds contained Cd and 22 feeds as at detectable levels; however, Cd did not exceed MRL values in any feed analyzed. As levels in lactating cow (2.45 ppm) and lamb fattening (2.98 ppm) feeds from Eastern Anatolia provided in July, and Pb in lactating cow feed (5.59 ppm) collected from Agean Region in October exceeded MRL of the TMARA instruction. Similarly, Hg in one of the lamb fattening feeds (0.15 ppm) coming from Aegean Region in July and one of the cattle fattening feeds (0.16 ppm) obtained from Eastern Anatolia in July were found over the MRL; while, a total of 50 samples contained Hg only at detectable levels.

Pb is observed most frequently in cereals and since it is accumulated on the shell of grains through dusts and the rain, it is usually higher in bran compared to the whole grain (Ocker and Brüggemann 1991). It may also be high where industrial facilities and traffic jam are intensified (Mert et al. 1993). In the present study, high Pb figures exceeding MRL in October's lactating cow feed may have caused by bran used in compound feeds and air pollution during autumn. 60% of samples containing Pb, Hg, As over MRL were obtained from the feeds coming Eastern Anatolia. This was found to be interesting since industrialization level in Eastern Anatolia is not comparable to other parts of the country.

Positive cases of 2, 19, 17, 15, 10, 2 and 18 Pb toxicity were reported for each year by Minnesota Diagnostic Laboratory between 1994 and 2000, respectively. The ratio of lead poisoning differed between 0.5 to 8.0% in all samples submitted by dairy farms to the laboratory during the same period (Olson et al. 2002).

In addition to wastes of different industrial branches such as electrochemistry, paint, medicine, paper, metallurgy, fungicides are other important sources of Hg and Hg containing fungicides are widely used for cereals (Şanlı 1976).

About 5 to 20% of total Cd consumed by human beings originates from cereals and cereal products. However, Tietz and Brüggemann (1993) found Cd and Pb contents of 939 wheat and rye samples they examined to be lower than 0.005 ppm.

As contents of the crops may vary between 0.1 to 10.0 ppm depending on the pollution levels of soil and water. For example, as content of alfalfa is around 1.9; it is 0.3 ppm in wheat; while in sugar beat varies from 0.34 to 1.3, and changes in corn stover between 0.7-2.7. As, in foods higher than 3.5 ppm is accepted as toxic (Kurtoğlu and Coşkun 2001).

Quintozone and diazinon were detected in 5 and 2 samples, respectively. No pesticide residue was detected in all the remaining samples. Quintozone has applied as fungicide to cotton, peanut, sesame and anise; while, diazinon was utilized as insecticide and acaricide for sesame, cereals and cotton.

Since the instruction of TMARA does not contain any permitted MRL data for these two pesticides related to compound feeds, no comparison has been made.

In a study conducted in India on compound feeds and feedstuffs, Unnikrishan et al. (1998) found HCH levels to be between 7.6-119.7 ppb, DDT up to 49.1 ppb and endosulfan up to 10 ppb. DDT was observed most frequently in peanut meal.

Lovell et al. (1996) analyzed 545 compound feed samples collected by FDA between 1989-1994. They found pesticide residues only at detectable levels in 457 samples; but, none of them exceeded Food and Drug Administration (FDA) limits.

In a similar work carried out by Prasad et al. (2002) on the organochlorine pesticides contaminating ruminant feeds such as cereals, oil seed meals, brans, leguminous and none leguminous forages, the levels of various residues ranked as HCH > endosulfan > heptachlor > DDT > aldrin. In contrast, no DDT was found in all samples analyzed in the present study.

Depending on the data discussed above it can be concluded that, 1) Feed manufacturers are quite careful in terms of aflatoxin contamination. Thus, it can be claimed that compound feeds produced in Turkey are safe for aflatoxins. 2) DDT was not found in any feed analyzed. 3) Presence of 2 pesticide residues in only 7 samples proves that there is no serious pesticide residue problem in compound feeds produced throughout the country. 4) Similarly, heavy metal pollution was observed in only 3 samples indicating that heavy metal pollution in compound feeds produced in Turkey is low. 5) Instruction about Undesired Materials in Feeds prepared by Ministry of Agricultural and Rural Affairs is insufficient for compound feeds. Since compound feeds are the last rings of feed chain directly reaching the animal, the instruction should be rearranged to cover compound feeds.

Further studies related to residues in feeds covering some other substances such as polychlorinated biphenyls (PCBs) and mycotoxins other than aflatoxins as well as various rings of the feed industry including feed and animal produces, logistics and dealers.

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