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Determination of natural adverse factors (aflatoxins, nitrate, nitrite, tannin, and sodium chloride) in some cage bird feeds

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Abstract: This study was carried out to determine the levels of aflatoxins, nitrate, nitrite, tannin and sodium chloride in some cage bird feeds sold in Ankara, Turkey. A total of 60 feed samples (20 each of budgerigar, canary and parrot feed) were analysed. Aflatoxin analyses by HPLC, nitrate, nitrite and tannin analyses spectrophotometrically and sodium chloride analyses by a method based on colour reaction were performed. Total aflatoxin was found to be 2.44-34.30 ppb in 11 budgerigar feeds, 0.91-16.75 ppb in 8 canary feeds and 1.43-19.29 ppb in 10 parrot feeds. Nitrite (0.00-6.19 ppm), nitrate (1.31-25.55 ppm), tannin (0.04-0.74%), and sodium chloride (0.05-0.44%) were found in all feeds. As a result, it was concluded that the level of AFB1 in only 1 budgerigar feed might pose a risk to the health of cage birds, while the levels of nitrate, nitrite, tannin and sodium chloride might not cause any health problems.

Keywords: Aflatoxin, cage bird feed, nitrate-nitrite, sodium chloride, tannin

Bazı kafes kuşu yemlerinde doğal olumsuzluk faktörlerinin (aflatoksin, nitrat, nitrit, tanen ve sodyum klorür) belirlenmesi

Özet: Bu çalışma, Ankara'da satılan bazı kafes kuşu yemlerinde aflatoksin, nitrat, nitrit, tanen ve sodyum klorür düzeylerini belirlemek amacıyla gerçekleştirildi. Toplam 60 yem örneği (muhabbet kuşu, kanarya ve papağan yeminin her birinden 20 adet) analiz edildi. HPLC ile aflatoksin analizleri, spektrofotometrik olarak nitrat, nitrit ve tanen analizleri ve renk reaksiyonuna dayalı bir yöntemle sodyum klorür analizleri yapıldı. Toplam aflatoksin 11 muhabbet kuşu yeminde 2.44-34.30 ppb, 8 kanarya yeminde 0.91-16.75 ppb ve 10 papağan yeminde 1.43-19.29 ppb olarak bulundu. Tüm yemlerde nitrit (0.00-6.19 ppm), nitrat (1.31-25.55 ppm), tanen (%0.04-0.74) ve sodyum klorür (%0.05-0.44) bulundu. Sonuç olarak, sadece 1 muhabbet kuşu yemindeki AFB1 düzeyinin kafes kuşlarının sağlığı için risk oluşturabileceği, nitrat, nitrit, tanen ve sodyum klorür düzeylerinin ise herhangi bir sağlık sorununa neden olmayabileceği sonucuna varıldı.

Anahtar kelimeler: Aflatoksin, kafes kuşu yemi, nitrat-nitrit, sodyum klorür, tanen

Introduction

With the widespread use of cage bird breeding, the frequency of veterinarians encountering care, nutrition and health problems of these animals is increasing (Salt et al. 2000). While veterinarian is evaluating the condition of sick animals, it is also very important to evaluate the mycotoxins, nitrate, nitrite, tannin and sodium chloride which are naturally present or may occur in feed or foodstuffs as well as infectious agents in terms of correct diagnosis and appropriate treatment (Barug et al. 2003; Basmacioğlu and Ergül 2003; Blake 2008).

Aflatoxins constitute the most important group among mycotoxins. Aflatoxins are synthesised by *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and various toxigenic *Aspergillus* and some *Penicillium* and

Rhizopus moulds (Rustom 1997; Thompson and Henke 2000; Mishra and Das 2003; Nicholas 2003; O'keeffe 2003; Williams et al. 2004; Akande et al. 2006). It is reported that feed is contaminated with mycotoxins at a higher rate when compared to about 25 per cent of the agricultural products produced every year in the world (Akande et al. 2006; Dorina et al. 2008; Reddy and Waliyar 2009). Poisoning (mycotoxicosis) of acute or chronic character occurs in humans and animals eating food and feed contaminated with these toxins (Wood 1992; Nizamlioglu and Gözü 1996; Anon 2008a; Becer 2008).

Generally, birds are more sensitive than mammals. Young birds are more susceptible than adults. In poultry, the most susceptible species are ducklings and turkeys. Mortality due to aflatoxin

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exposure has been reported in migratory birds such as various duck species, Canada goose and crane (Robinson et al. 1982; Anon 2008b; Diaz et al. 1995).

Nitrate and nitrite are widespread in nature. Normally, fresh plants contain a few grams of nitrate per kilogramme. The nitrate and nitrite content of cereal grains ranges from 0.5 to 18 mg/kg depending on the species and growth condition (Diaz et al. 1995). Acute or chronic poisoning occurs when animals ingest excessive amounts of nitrate and nitrite accumulated in plants and groundwater for different reasons (Stoltenow and Lardy 1998; Casteel and Evans 2004; Nicholson 2007). The intensive and excessive use of nitrogen fertilisers in agriculture on the grounds that they increase yields leads to excessive nitrate accumulation in some plant species and groundwater. As a result of this accumulation, acute or chronic disorders occur in both humans and animals (Alçiçek and Başlar 1995).

Tannins are nitrogen-free, polyphenolic, amorphous compounds found naturally in plants. Tannins are degraded in the digestive system of animals and transformed into phenol structures such as gallic acid, pyrogallol and pyrocatechol which are more toxic than tannins (Schofield et al. 2001; Üstün and Aydın 2007). They form complex compounds with proteins, amino acids, polysaccharides, minerals and some vitamins and reduce their digestibility (Hagerman et al. 1992; Akar et al. 1994; Schofield et al. 2001).

If tannins are present in excessive amounts in feeds, they cause toxic effects in animals that consume these feeds or cause developmental retardation and decreased feed utilisation. In addition, tannins, which are known as a strong liver and kidney poison, can also cause carcinogenic effects. The toxicity of tannins is due to their astrengenic, irritant and haemolysing effects (Price et al. 1993; Reed 1995; Üstün and Aydın 2007).

Sodium chloride, which is a basic nutrient, is normally found in animal feeds at 0.5-1%. Due to its attractive and less irritating flavour, it can sometimes be taken in excessive amounts by animals and can cause poisoning. Poultry are very sensitive to sodium chloride poisoning (Kaya and Akar 2002).

There are not enough studies on the levels of aflatoxin, nitrate, nitrite, tannin and sodium chloride in cage bird feeds. In recent years in Turkey, the care, feeding and treatment of birds have been mainly included in the area of responsibility of veterinary medicine. Therefore, it is necessary to carry out toxicological analyses of bird feeds in terms of various

residues and contaminants in terms of preventive medicine and diagnosis. When evaluated from this point of view, it is thought that the levels found as a result of this study will be a source for further research on the subject.

Materials and methods

Sampling

In this study, 20 feed samples of 3 different bird species, namely budgerigar, canary and parrot feed, and 60 feed samples in total were analysed. Feed samples were collected from pet shops, bird shops and markets in Ankara. Nitrate-nitrite, tannin and sodium chloride analyses of the feed samples were performed in the Application Laboratories of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, and aflatoxin analyses were performed in a laboratory accredited by the Turkish Accreditation Agency.

Aflatoxin analysis

Aflatoxin analyses were performed by HPLC according to the method reported by Stroka et al. (2003). The method was based on extraction of the analysed sample with acetone/water solvent mixture; clean-up with immuno-affinity column (IAK) containing monoclonal antibodies specific for aflatoxins B1, B2, G1, G2; post-column electrochemical bromine derivatisation and determination of aflatoxins B1, B2, G1, G2 by reverse phase liquid chromatography (RP-HPLC) with fluorescence detector.

For the determination of nitrate and nitrite levels in feeds, the method reported by Sen and Donaldson (1978), modified by Yavuz (1992) and Oruç and Ceylan (2001) and applied by Becer (2008) was used. The method is based on the extraction of nitrate and nitrite from homogenised or ground samples with distilled water. According to the method, the extracted nitrate was reduced to nitrite by passing through a cadmium column. The nitrite content at acidic pH was measured by a colorimetric method by adding N-(1-Naphthyl) ethylene diamine (NED) dihydrochloride mixed with sulfanilic acid as a colour reagent and converted into red azo dye.

Nitrite analysis

Five ml of the sample filtrate was measured and transferred to a 50 ml measuring cylinder. It was added 9 ml of ammonium chloride buffer, 5 ml of 60% acetic acid, 5 ml of sulfanilamide, 2 ml of NED. It was completed to 50 ml with distilled water. A blind sample was prepared in the same way using distilled water instead of filtrate. The prepared sample and

blind were kept in the dark for 25 min. Absorbance was read against the blind at a wavelength of 550 nm in a spectrophotometer.

Nitrate analysis

Two ml of the sample filtrate was measured and transferred to a 50 ml measuring cylinder. 5 ml of ammonium chloride buffer was added and mixed. Before nitrate determination, nitrate was reduced to nitrite by passing through the activated cadmium reduction column at a rate of 3-5 ml/min. After each sample filtrate passed through the column, the column was washed with 15 ml distilled water. To the sample passing through the column, 5 ml 60% acetic acid, 5 ml sulfanilamide, 2 ml NED were added respectively. It was completed to 50 ml with distilled water. A blind sample was prepared in the same way using distilled water instead of filtrate. The prepared sample and blind were kept in the dark for 25 min. Absorbance was read against the blind at a wavelength of 550 nm in a spectrophotometer.

Tannin analysis

Tannins in the feeds were determined according to the spectrophotometric method reported by Horwitz (1970) and Lepper (1950). Tannin, together with sodium carbonate, was coloured blue with Folin-Denis reagent. The amount of tannin was determined by measuring the blue coloured compound in a spectrophotometer at 760 nm. The results were given as percentage.

Sodium Chloride analysis

Sodium chloride level was determined according to USP-NF 1985. 5 g of feed sample was mixed with some distilled water in a mixer and the mixture was transferred into a 500 ml flask; the sample residues in the mixer and funnel were washed with distilled water. The mixture was heated at 45°C for 25-30

min to dissolve the salt and precipitate albumin, the volume was increased to 500 ml with distilled water, cooled and filtered. The filtrate was taken 50 ml, 1 ml of the indicator (potassium chromate 5% prepared in water) was added and titrated to brick red colour with 0.1 N AgNO₃ solution. A blind (with 5 ml of water) was made in the same way. The volume of 0.1 N AgNO₃ consumed was determined. The results were given as percentage.

Statistics

Statistical evaluation of the data obtained at the end of the study was performed with SPSS 17 statistical package programme. After determining whether total aflatoxin, nitrate-nitrite, tannin and sodium chloride levels were normally distributed or not by Kolmogorov-Smirnov test, the difference in the means between the normally distributed levels in terms of the specified variables was determined by one-way analysis of variance, and the groups showing differences were determined by Duncan least significant difference method.

Results

According to the results of the analyses, total aflatoxin levels (Table 1) were 2.44-34.30 ppb in 11 budgerigar feeds (Table 2), 0.91-16.75 ppb in 8 canary feeds (Table 3) and 1.43-19.29 ppb in 10 parrot feeds (Table 4). According to aflatoxin types, AFB₁ was between 2.44-28.14 ppb in 11 budgerigar feeds, 0.91-15.5 ppb in 8 canary feeds and 1.43-18.5 ppb in 10 parrot feeds; AFB₂ was between 0.29-1.66 ppb in 9 of the budgerigar feeds, 0.27-1.25 ppb in 3 of the canary feeds and 0.25-1.44 ppb in 5 of the parrot feeds; AFG₁ was detected at levels between 3.9-8.13 ppb in 3 of the budgerigar feeds and 1.46 ppb in 1 of the parrot feeds and AFG₂ was detected at levels of 0.6 ppb in only 1 budgerigar feed.

Table 1. Aflatoxin levels in all feeds (ppb).

Aflatoxins	Budgerigar X ± Sx	Canary X ± Sx	Parrot X ± Sx	General X ± Sx
AFB ₁ min - max	5.41 ± 1.65 0.00 – 28.14	1.90 ± 0.82 0.00 – 15.50	3.48 ± 1.25 0.00 – 18.50	3.60 ± 0.75 0.00 – 28.14
AFB ₂ min - max	0.34 ± 0.12 0.00 – 1.66	0.09 ± 0.06 0.00 – 1.25	0.21 ± 0.10 0.00 – 1.44	0.21 ± 0.06 0.00 – 1.66
AFG ₁ min - max	0.80 ± 0.47 0.00 – 8.13	ND	0.07 ± 0.07 0.00 – 1.44	0.29 ± 0.16 0.00 – 8.13
AFG ₂ min - max	0.03 ± 0.03 0.00 – 0.60	ND	ND	0.01 ± 0.01 0.00 – 0.60
Toplam AF min - max	6.58 ± 2.06 0.00 – 34.30	1.99 ± 0.88 0.00 – 16.75	3.76 ± 1.33 0.00 – 19.29	4.11 ± 0.88 0.00 – 34.30

ND: No detectable levels were found.

Table 2. Aflatoxin levels in budgerigar feeds.

Sample Number	Result (ppb)				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total
1	14.76	1.66	3.9	-	20.32
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	13.79	0.6	-	-	14.39
8	2.53	0.29	-	-	2.82
9	2.95	0	-	-	2.95
10	6.48	0.38	-	-	6.86
11	-	-	-	-	-
12	7.14	0.42	-	-	7.56
13	9.41	0.47	-	-	9.88
14	-	-	-	-	-
15	-	-	-	-	-
16	28.14	1.66	3.9	0.6	34.3
17	-	-	-	-	-
18	12.7	0.8	-	-	13.5
19	7.95	0.55	8.13	-	16.63
20	2.44	-	-	-	2.44

- : No detectable levels were found.

Table 3. Aflatoxin levels in canary feeds.

Sample Number	Result (ppb)				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total
1	-	-	-	-	-
2	2.78	-	-	-	2.78
3	-	-	-	-	-
4	4.38	0.27	-	-	4.65
5	-	-	-	-	-
6	15.5	1.25	-	-	16.75
7	1.77	-	-	-	1.77
8	-	-	-	-	-
9	4.93	-	-	-	4.93
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-

Sample Number	Result (ppb)				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total
13	-	-	-	-	-
14	-	-	-	-	-
15	0.91	-	-	-	0.91
16	2.65	-	-	-	2.65
17	-	-	-	-	-
18	-	-	-	-	-
19	5.05	0.3	-	-	5.35
20	-	-	-	-	-

- : No detectable levels were found.

Table 4. Aflatoxin levels in parrot feeds.

Sample Number	Result (ppb)				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total
1	-	-	-	-	-
2	-	-	-	-	-
3	11.69	1.23	-	-	12.92
4	10.21	0.51	-	-	10.72
5	-	-	-	-	-
6	-	-	-	-	-
7	2.18	-	-	-	2.18
8	3.29	-	-	-	3.29
9	-	-	-	-	-
10	1.43	-	-	-	1.43
11	-	-	-	-	-
12	-	-	-	-	-
13	18.5	0.79	-	-	19.29
14	-	-	-	-	-
15	1.58	-	1.46	-	3.04
16	-	-	-	-	-
17	14.6	1.44	-	-	16.04
18	-	-	-	-	-
19	3.68	0.25	-	-	3.93
20	2.52	-	-	-	2.52

- : No detectable levels were found.

In addition, an example of a negative (Figure 1) and positive chromatogram (Figure 2) of the analysed samples and an example of the chromatogram of the standard (Figure 3) are given.

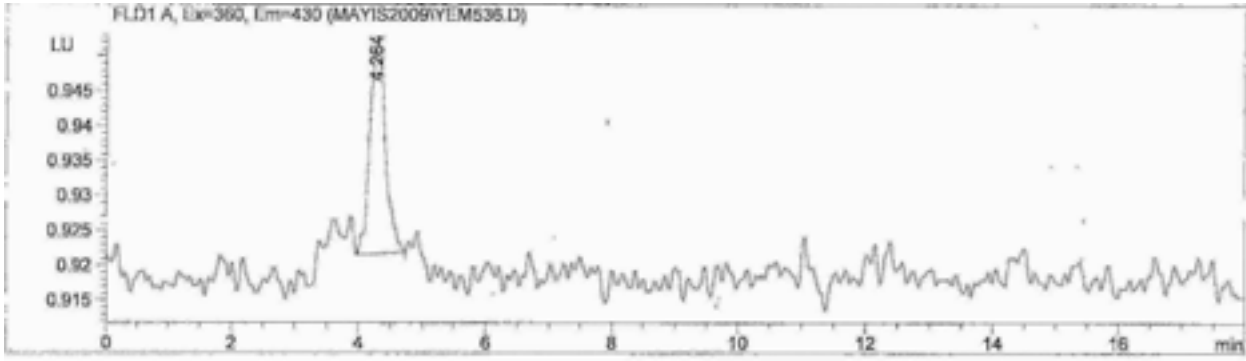


Figure 1. Negative feed sample chromatogram.

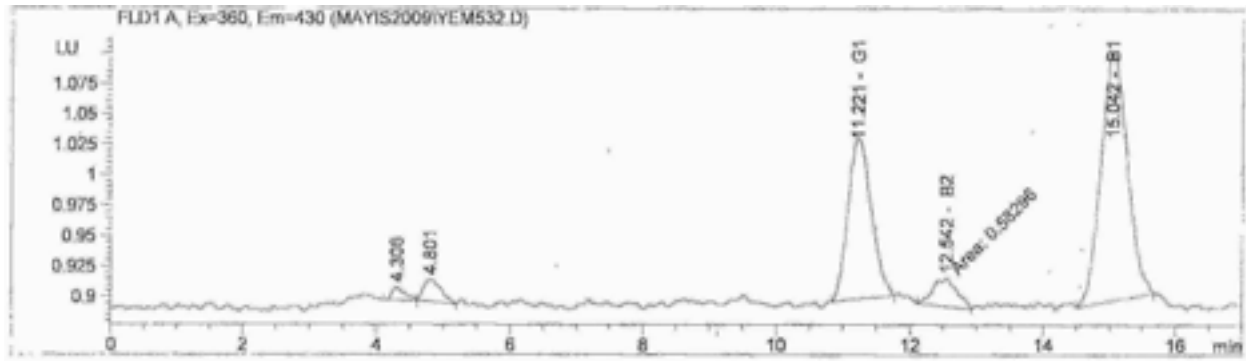


Figure 2. Positive feed sample chromatogram.

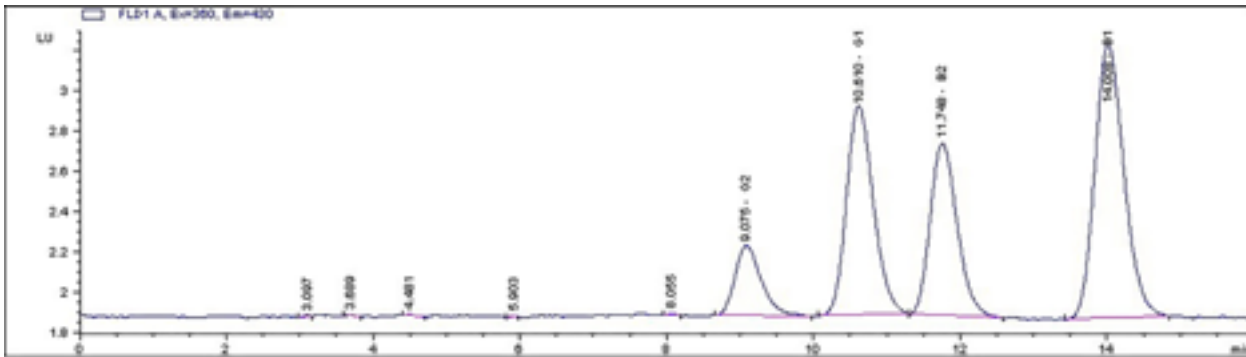


Figure 3. Chromatogram of the standard.

Feed varieties were similar in terms of total aflatoxin, AFB1, AFB2, AFG1 and AFG2 levels ($p>0.05$).

As a result of the analyses, nitrite levels were detected between 0.48-6.19 ppm in 32 samples and nitrate levels were detected between 1.31-25.55 ppm in all samples. According to the feed types, nitrate levels were 7.95 ± 1.33 ppm (1.31-25.55 ppm) in

budgerigar feeds, 7.47 ± 0.60 ppm (2.62-13.76 ppm) in canary feeds, 6.95 ± 0.60 ppm (1.97-12.45 ppm) in parrot feeds; nitrite levels were 1.48 ± 0.41 ppm (0.00-6.19 ppm) in 10 samples in budgerigar feeds, 0.83 ± 0.28 ppm (0.00-3.81 ppm) in 8 samples in canary feeds and 2.21 ± 0.38 ppm (0.00-4.76 ppm) in 14 samples in parrot feeds (Table 5).

Table 5. Nitrate, nitrite, tannin and sodium chloride levels in feeds.

Substance	n	Budgerigar X ± Sx	Canary X ± Sx	Parrot X ± Sx	General* X ± Sx	P
Nitrite (ppm) min - max	20	1.48 ± 0.41 ^{ab} 0.00 – 6.19	0.83 ± 0.28 ^b 0.00 – 3.81	2.21 ± 0.38 ^a 0.00 – 4.76	1.51 ± 0.22 0.00 – 6.19	x
Nitrate (ppm) min - max	20	7.95 ± 1.33 ^a 1.31 – 25.55	7.47 ± 0.60 ^a 2.62 – 13.76	6.95 ± 0.60 ^a 1.97 – 12.45	7.46 ± 0.52 1.31 – 25.55	xxx
Tannin (percent) min - max	20	0.10 ± 0.007 ^a 0.04 – 0.15	0.12 ± 0.005 ^a 0.09 – 0.15	0.66 ± 0.013 ^b 0.52 – 0.74	0.29 ± 0.034 0.04 – 0.74	xx
Sodium chloride (percent) min - max	20	0.25 ± 0.018 ^a 0.11 – 0.44	0.11 ± 0.009 ^b 0.05 – 0.18	0.23 ± 0.015 ^a 0.09 – 0.35	0.19 ± 0.012 0.05 – 0.44	xxx

a, b: Differences between groups with different letters in the same row are significant.

x: p < 0.05 xx: p < 0.001 xxx: p > 0.05 *: n = 60

Tannin levels were found at ratios between 0.04-0.74% in all feeds. According to the feed types, 0.10±0.007% (0.04-0.15%) was found in budgerigar feeds, 0.12±0.005% (0.09-0.15%) in canary feeds and 0.66±0.013% (0.52-0.74%) in parrot feeds (Table 5).

Sodium chloride was found between 0.05-0.44% in all feeds. According to the feed types, 0.25±0.018% (0.11-0.44%) was found in budgerigar feeds, 0.11±0.009% (0.05-0.18%) in canary feeds and 0.23±0.015% (0.09-0.35%) in parrot feeds (Table 5).

In terms of nitrite, budgerigar feed was similar to canary and parrot feed. There was a significant difference between canary and parrot feeds (p<0.05). All groups were similar in terms of nitrate (p>0.05). In terms of tannins, budgerigar and canary feeds were similar; parrot feed was significantly different from these groups (p<0.001). In terms of sodium chloride, budgerigar and parrot feeds were similar (p>0.05), while there was a significant difference in canary feed (p<0.05).

Discussion and Conclusion

As a result of the scientific searches, a very limited number of studies with regard to the levels of aflatoxins, nitrate-nitrite, tannin and sodium chloride, which are natural adverse factors in cage bird feeds, were found.

In the study conducted by Oruç et al. (2001) by ELISA method in 22 bird feeds (12 budgerigars, 5 canary and 5 zoo bird feeds) in Bursa, total aflatoxin was found in 72.72% of the analysed feeds and it was determined to be in the levels of 0.0-9.2 ppb. In this study than the study of Oruç et al. (2001), total aflatoxin was detected at a lower rate (48.3%) but at higher levels (0.00-34.30 ppb).

In a study conducted by Nizamlioğlu and Gözün (1996) by thin layer chromatography method in 12 different species and ages of poultry brought to the laboratory with suspicion of poisoning and in the feeds consumed, AFB1 has been detected in the feeds in levels of 2.5-25 ppb and it has been reported that pathological disorders due to aflatoxin are observed in the liver and kidneys of the animals as a result of necropsy. The levels of AFB1 found in this study (0.91-28.14 ppb) were similar to the levels (2.5-25 ppb) found according to the results of the analyses performed by the researchers.

Martins et al. (2003) have not detected aflatoxin in analysed 20 bird feeds (10 canary and 10 parrot feeds) for mycotoxins by HPLC method.

In a screening study conducted by Henke et al. (2001), aflatoxins ranging from 0 to 2780 ppb were detected in 142 wild bird feed samples consisting of corn, millet, sunflower seeds and cereal grains collected from different regions of Texas by Aflatest kits and fluorimetric method and it was reported that 17% of these feeds contained aflatoxins above 100 ppb. The aflatoxin levels found in this study (0.00-34.30 ppb) were much lower than the levels found by Henke et al.

In a study by Maia and Pereira Bastos de Siquiera (2002), AFB1 was detected in 26.7% of 30 bird feeds by thin layer chromatography method with a mean of 110 ppb in 26.7% of the bird feeds. It has been reported that all feeds in which aflatoxin is detected contain peanuts. When compared with the results of the studies here, the AFB1 levels found by the researchers (mean 110 ppb) were much higher than the mean AFB1 levels found in this study (3.60±0.75).

The contamination of feed and feed raw materials with aflatoxins varies significantly according to

feed content, countries, regions, seasons and time due to the preparatory factors causing toxin formation. For this reason, there were significant differences between the findings of the studies.

There is no data on aflatoxin tolerance levels in cage bird feeds. The levels obtained as a result of the research were evaluated according to the Communiqué on undesirable substances in feed numbered 2005/3 published in the Official Gazette dated 5 February 2005 and numbered 25718 and on the amendment of the Communiqué on undesirable substances in feed published in the Official Gazette dated 11 June 2008 and numbered 26903 within the framework of European Union harmonisation laws. According to this communiqué, the maximum amount of AFB1 that can be found in feedstuffs is reported as 0,02 mg/kg (20 ppb). Similarly, the Advisory Committee on Animal Feedingstuffs in the UK reported the maximum amount of AFB1 in feedstuffs for wild birds as 0.02 mg/kg. As a result of the study, it was determined that only 1 sample of budgerigar feed exceeded the specified levels.

In a study conducted by Oruç et al. (2001) on a total of 22 bird feeds including 12 budgerigars, 5 canaries and 5 zoo birds, 0.0-3.1 ppm nitrate and 0.0-1.3 ppm nitrite were found in the feeds. When compared with the results of this study (nitrate 1.35-25.5 ppm and nitrite 0.00-6.19 ppm), it was found that the levels found by the researchers were lower.

In a study conducted by Atef et al. (1991) in cockerels, it was determined that growth was slowed down, methaemoglobinaemia and changes in erythrocyte, glutamic pyruvic transaminase, creatinine and urea levels developed in both of the two different groups given 4.2 g/kg sodium nitrate and 1.7 g/kg sodium nitrite with feed. Therefore, it was stated that nitrate and nitrite might play a role in the aetiology of liver, kidney and immune system related diseases in poultry.

The levels found here were similar to the levels of nitrate and nitrite (0.5-18 ppm) normally found in cereal grains reported by Diaz et al. (1995).

Akar et al. (1994), in a study on tannin content in various feed materials, found that tannin content in feed materials ranged between 0 and 5.70 per cent and concluded that tannin content at these levels might cause health problems in poultry and adversely affect feed utilisation. The results obtained in this study (0.04-0.74%) were found to be lower than the results found by the researchers.

In a study conducted by Pour and Edriss (1997) in broiler chickens, it was reported that while no

adverse effects were observed in animals fed with feed containing up to 0.26% tannin, performance decreased in animals fed with feed containing tannin above this level.

Poultry are more sensitive to sodium chloride poisoning than other species. For these animals, safe and toxic levels of sodium chloride are very close to each other (Balnave 2006).

Berger (2006) reported that the ratio of sodium chloride in the feed of chicken, turkey, duck, goose, pheasant and quail should be between 0.25-0.5%.

Although there is no data on the sensitivity limits of cage birds to sodium chloride, it was concluded that the levels determined as a result of the analyses (0.05-0.44%) would not cause any health problems in cage birds since they were below the levels specified in the literature.

The levels of nitrate-nitrite, tannin and sodium chloride were evaluated in the light of the literature on poultry and it was concluded that the levels found were at levels that would not cause any health problems in animals.

Conclusions

The levels of nitrate-nitrite, tannin and sodium chloride detected in feeds do not seem to pose any risk to the health of cage birds, but the level of AFB1 found in only 1 budgerigar feed may pose a risk to the health of cage birds. However, the lack of sufficient studies on the natural adverse factors found in bird feeds and their effects on the health of birds makes it necessary to carry out new studies in order to better evaluate the issue and to determine legal limits. In particular, the high levels and poisoning cases detected in some studies show that it would be useful to screen bird feeds in terms of natural adverse factors at certain intervals.

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Declaration of competing interest: The authors would like to declare that there are no competing interests.

Ethics Committee Approval: This study is not related any experimental animal study, thus, is not required approval of Animal Experiments Local Ethics Committee.

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