Comparison of some mycotoxin concentration and prevalence in premium and economic class of adult dog foods

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ABSTRACT
The aim of this study determined the concentrations of total aflatoxin, ochratoxin-A, fumonisin-B1 and zearalenone in 40 different extruded dry type from premium (or with high price) (P-food) (n = 20) and economic (or with low price) (E-food) (n = 20) class foods with different nutritional content. According to ingredients, lamb meal (or by products) + cereal grain (LG-food), fish meal (or by products) + cereal grain (FG-food), chicken meal (or by products) + cereal grain (CG-food), and animal by-products + grain-free (GFree-food) (with potato or tapioca) were selected equal numbers for E-food and P-foods. Total aflatoxin was not detected in the GFree-dog foods and the FG-dog foods. The total aflatoxin concentrations of P-food and E-food were 1.30 ppb and 1.98 ppb in DM, respectively (P = 0.154). The fumonisin-B1 was detected in 10% of P-foods and 70% of E-foods (P < 0.001). The fumonisin-B1 concentration in GFree-food of dog was lower than those of other dog foods (P < 0.05). The highest fumonisin-B1 concentration of dog foods was in E-food of CG-food (3107 ppb in DM). The zearalenone concentration of GFree-food was lower than those of LG-food, FG-food and CG-food (P < 0.05). As a result, the total mycotoxins concentration of GFree-food (106 ppb in DM) were lower than those of other dog foods. The total mycotoxin concentration of E-food were higher than that of P-foods. The highest total mycotoxin concentration was in the E-food classes of CG-food. Therefore, zearalenone concentration of P-food was higher than that of E-food.

HIGHLIGHTS
• The total mycotoxin, total aflatoxin, ochratoxin-A and fumonisin-B1 concentration of the economic class dog food were higher than those of the premium class dog food.
• The highest total mycotoxin concentration was in the economic class dog food with chicken + grain.
• Ochratoxin-A has not been detected in any of the premium class dog foods.
• Economic class of grain-free dog foods contained very low concentrations of fumonisin-B1 and zearalenone mycotoxins, while premium class of they contained none.

Introduction
Mycotoxins are secondary metabolites produced by fungi especially those belonging to the genus Aspergillus, Penicillum and Fusarium. Mycotoxins are secondary fungal metabolites that produce toxic effects on animals and humans. Depending on classification, 300–400 mycotoxins are known to date (Agriopoulou et al. 2020; Streit et al. 2012). Not all fungi are capable of producing mycotoxins; so-called toxigenic only produce them. The most common mycotoxins in feedstuffs include aflatoxins, fumonisins, ochratoxin-A, zearalenone and trichothecene deoxynivalenol, T-2 toxin and HT-2 toxin. Mycotoxins are secondary metabolites produced by filamentous fungi that can contaminate cereal grain, often due to improper grain storage (Singh et al. 2018). Mycotoxins contaminate cereal grains worldwide and their presence in pet foods has posed a potential health threat to pet animals. Dry-pellet dog foods contain higher amounts of cereal grains than those of wet-canned dog foods. These high cereal grain content potentially can be cause to high mycotoxin levels. Grains, especially corn, sorghum, rice, wheat, oats, barley, and millet, in dry dog foods are the most likely source of mycotoxin contamination (Tegzes et al. 2019; Martínez-Martínez et al. 2021). Aflatoxins, Ochratoxin-A
and *Fusarium* mycotoxins have been detected in both the raw components and the final product (extruded food) of pet food worldwide (Tegzes et al. 2019; Castaldo et al. 2019). Aflatoxin, a hepatotoxin and carcinogen, has caused several outbreaks of food poisoning in dogs, and in many countries, a certain level of aflatoxin content in pet food has been given importance (FDA 2021). When dogs ingest extruded dog food made with aflatoxins, they are absorbed in the duodenum and bind to plasma albumin and proceed to be transported through the bloodstream (Tessari et al. 2010). These mycotoxins have various harmful cytotoxic mechanisms (Singh et al. 2018). The European Union has set a legal limit of 20 ppb for aflatoxin-B1, which makes up most of the total aflatoxins, in animal feed (FAO 2004) and has published guidelines for deoxynivalenol and fumonisin (EU 2003). *Fusarium* mycotoxins, including ochratoxin-A, trichothecenes, zearalenone and fumonisins, may have chronic poisoning effects on the health of pet animals (Leung et al. 2006). The clinical effects of mycotoxins vary with the type, concentration and frequency of exposure. Some mycotoxins cause morbidity and mortality both acutely due to high-dose exposures and chronically after long-term low-dose exposures. Effects may include acute toxicity such as anorexia, depression, gastrointestinal bleeding, jaundice, or acute liver injury manifesting as seizures (Tegzes et al. 2019). Chronic diseases such as liver and kidney fibrosis, infections from immunosuppression, and cancer have been associated with low-dose chronic mycotoxin exposure (Boermans and Leung 2007). In a clinical study, a mycotoxin combination containing aflatoxin-B1 and B2, fumonisin-B1 and B2, ochratoxin-A and zearalenone was found to induce immunotoxicity on canine peripheral blood mononuclear cells (Singh et al. 2018). Therefore, the potential for mycotoxic contamination in pet food poses a serious health threat (Boermans and Leung 2007). As it can be understood, determining the mycotoxin levels in dog foods and determining which food group is higher is important for dog health. Determining the levels of mycotoxins, which are encountered in dogs and may be the cause of some metabolic diseases for which only symptomatic treatment is used, is important in premium and economic class foods containing different protein (lamb, fish and chicken meal) and carbohydrate (grain or potato/tapioca flour) sources offered for sale will guide researchers and animal owners. The aim of this study determined the concentrations of total aflatoxin, ochratoxin-A, fumonisin-B1 and zearalenone in 40 different brand extruded dry type with different nutritional content (cereal or grain-free), which were economic and premium class foods, produced commercially for adult dogs.

Materials and methods

The samples of dog food

In the study, premium (or with high price) (P-food) and economic (or with low price) classes (E-food) of dry type foods, produced for adult dogs, were selected. Equal numbers of P-food and E-food were obtained as 40 different commercial dog foods according to content features: lamb meal (or by products) + cereal grain (LG-food) (n = 10), fish meal (or by products) + cereal grain (FG-food) (n = 10), chicken meal (or by products) + cereal grain (CG-food) (n = 10) and animal by-products + grain-free (GFree-food) (with potato or tapioca) (n = 10). The dog foods were not obtained from the market or veterinary clinic, but directly from large companies that sell and distribute many different dog foods from Local Company (Istanbul, Turkey). Thus, after the production, the food was supplied from the first sales step. Mycotoxins analyzes were carried out after the dog foods were ground to a diameter of 1 mm (IKA, Germany). Each food sample was analysed in triplicate for mycotoxins. The results were given as ppb in dry matter (DM) of dog foods.

Total aflatoxin analyses

Total aflatoxin concentrations in dog foods were determined using a microplate reader ELISA Kit (Elabscience Biotechnology Inc., Total Aflatoxin, Catalog No: E-TO-E006, USA). The 2 g of the grounded sample was weighed into the 50 mL centrifuge tube. The 5 mL of 70% methanol was added to the tube and centrifuged at 4000 x g for 10 minutes. The 0.5 mL of supernatant was taken to another centrifuge tube, and added 0.5 mL of deionised water. The 50 μL of this sample and total aflatoxin standards (0, 0.02, 0.04, 0.08, 0.16 and 0.32 ppb) were tested in microplate wells in duplicate. The 50 μL of HRP conjugate was added to each well, and then 50 μL of antibody working solution was added. The microplate, covered with plate sealer, was incubated for 30 minutes at 25 °C in shading light. After the liquid in wells was removed, 300 μL of wash buffer immediately was added. This washing process was repeated for five times, 30 second intervals/time. The 50 μL of substrate reagent A and 50 μL of substrate reagent B were added to each well, respectively. The microplate was
incubated at 25°C for 15 minutes in shading light. At the end of time, the 50 μL of stop solution was added to each well and mixed thoroughly. The optical density of each well was determined at 450 nm with a microplate reader. Calculation was made according to the curve of total aflatoxin standard ($R^2 = 0.9395; y = -217.18x + 75.366$).

**Zearalenone analyses**

Zearalenone concentrations in dog foods were determined using a microplate reader ELISA Kit (Elabscience Biotechnology Inc., Zearalenone, Catalog No: E-TOE002, USA). The 2 g of grounded sample was weighed into the 50 mL centrifuge tube. The 8 mL of 90% methanol was added to the tube and centrifuged at 4000x g for 10 minutes. The 0.5 mL of supernatant was taken to another centrifuge tube, and added 1 mL of 90% methanol. The 0.25 mL of liquid was taken to micro-centrifuge tube, and fully mixed with 1 mL of deionised water. The 50 μL of this sample and zearalenone standards (0, 0.3, 0.9, 2.7, 8.1 and 24.3 ppb) were tested in microplate wells in duplicate. The 50 μL of HRP conjugate was added to each well, and then 50 μL of antibody working solution was added. The microplate, covered with plate sealer, was incubated for 30 minutes at 25°C in shading light. After the liquid in wells was removed, 300 μL of wash buffer immediately was added. This washing process was repeated for five times, 30 second intervals/time. The 50 μL of HRP conjugate was added to each well. The microplate was incubated at 37°C for 15 minutes in shading light. The before-mentioned washing process was repeated. The 50 μL of substrate reagent A and 50 μL of substrate reagent B were added to each well, respectively. The microplate was incubated at 37°C for 30 minutes in shading light. The 50 μL of stop solution was added to each well and mixed thoroughly. The optical density of each well was determined at 450 nm with a micro plate reader. Calculation was made according to the curve of ochratoxin-A standards ($R^2 = 0.923; y = -0.7614x + 68.785$).

**Fumonisin-B1 analyses**

Fumonisin-B1 concentrations in dog foods were determined using a microplate reader ELISA Kit (Elabscience Biotechnology Inc., Fumonisin FB1, Catalog No: E-TOE020, USA). The 1 g of grounded sample was weighed into the 50 mL centrifuge tube. The 5 mL of deionised water was added to the tube and centrifuged at 4000 xg for 10 minutes. The 0.1 mL of supernatant was taken to another centrifuge tube, and added 0.9 mL of reconstitution buffer. The 50 μL of this sample and fumonisin-B1 standards (0, 1, 3, 9, 27 and 81 ppb) were tested in microplate wells in duplicate. The 50 μL of HRP conjugate was added to each well, and then 50 μL of antibody working solution was added. The microplate, covered with plate sealer, was incubated for 30 minutes at 25°C in shading light. After the liquid in wells was removed, 300 μL of wash buffer immediately was added. This washing process was repeated for five times, 30 second intervals/time. The 50 μL of substrate reagent A and 50 μL of substrate reagent B were added to each well, respectively. The microplate was incubated at 25°C for 15 minutes in shading light. At the end of time, the 50 μL of stop solution was added to each well and mixed thoroughly. The optical density of each well was determined at 450 nm with a micro plate reader. Calculation was made according to the curve of fumonisin-B1 standards ($R^2 = 0.9716; y = -0.7214x + 65.359$).

**Ochratoxin-A analyses**

Ochratoxin-A concentrations in dog foods were determined using a microplate reader ELISA Kit (Elabscience Biotechnology Inc., Ochratoxin A, Catalog No: E-TOE001, USA). The 2 g of grounded sample was weighed into the 50 mL centrifuge tube. The 10 mL of 70% methanol was added to the tube and centrifuged at 4000 x g for 10 minutes. The 0.75 mL of supernatant was taken to another centrifuge tube, and added 1 mL of 0.1 M NaHCO₃ solution. The 50 μL of this sample and ochratoxin-A standards (0, 1, 3, 9, 27, and 81 ppb) were tested in microplate wells in duplicate. The 50 μL of antibody working solution was added to each well. The microplate, covered with plate sealer, was incubated for 30 minutes at 37°C in shading light. After the liquid in wells was removed, 300 μL of wash buffer immediately was added. This washing process was repeated for five times, 30 second intervals/time. The 50 μL of HRP conjugate was added to each well. The microplate was incubated at 37°C for 30 minutes in shading light. At the end of time, the 50 μL of stop solution was added to each well and mixed thoroughly. The optical density of each well was determined at 450 nm with a micro plate reader. Calculation was made according to the curve of ochratoxin-A standards ($R^2 = 0.923; y = -0.7614x + 68.785$).
Statistical analysis

Equal numbers of P-food (n = 20) and E-food (n = 20) were obtained as 40 different commercial dog foods according to content features [(LG-food; n = 10), (FG-food, n = 10), (CG-food, n = 10) and (GFree-food, n = 10)]. Statistical analysis of mycotoxin analysis data, which was performed in triplicate from each food sample, was performed.

Statistical analyzes of the raw data obtained from the study were made using the SPSS 17.0 package program. The prevalence of mycotoxins in dog foods was determined by the chi-square test. Mycotoxin concentrations in dog foods were determined by two-way analysis of variance according to food class and food content. When the significance was determined, the Tukey Multiple Range Test, one of the Multiple Comparison Tests, was applied. Values below the statistical significance level of 0.05 (P < 0.05) were taken.

Results

Total aflatoxin in commercial dog foods

Total aflatoxin was not detected in any of the GFree-dog foods and the FG-dog foods. However, total aflatoxin was detected in 90% and 70% of CG-food and LG-food, respectively. Total aflatoxin mean was detected in 90% of the premium foods and economy class foods. The total aflatoxin concentrations of premium and economic classes of dog foods with different ingredients ranged from 0.86 ppb to 2.35 ppb in DM (P = 0.827). The total aflatoxin concentrations of LG-food (0.89 ppb) and CG-food (2.06 ppb) were higher than those of FG-food (0.0 ppb) and GFree food (0.0 ppb) (P < 0.05). The total aflatoxin concentrations of premium and economic class dog foods were 1.30 ppb and 1.98 ppb in DM, respectively (P = 0.154) (Table 1).

Ochratoxin-a in commercial dog foods

According to the food ingredients, ochratoxin-A was detected in 10% of the LG-food, FG-food, CG-food and GFree-foods. Ochratoxin-A did not have premium class foods, but had 20% of economy class foods (P < 0.05). The ochratoxin-A concentrations of premium classes of LG-food, FG-food, CG-food and GFree-food were 0 ppb. But ochratoxin-A concentrations of economic classes of LG-food, FG-food, CG-food and GFree-food were 12.75, 71.67, 41.19 and 40.74 ppb in DM, respectively. The ochratoxin-A content of economic dog foods was higher that of premium dog foods (P < 0.05) (Table 2).
Fumonisin-B1 in commercial dog foods

According to the food ingredients, the fumanisin-B1 was detected in 60% of the studied LG-food. The fumonisin-B1 was detected in 40% of FG-food and 10% of premium dog foods and 70% of economic dog foods \( (P < 0.001) \). Fumonisin-B1 mycotoxin was detected in 40% of the total dog foods examined (Table 3).

### Table 2. Prevalence and concentrations of ochratoxin-A in commercial dog foods with different ingredients.

| Food ingredients | Food class | Concentration, ppb in DM | Prevalence, % |
|------------------|------------|--------------------------|---------------|
|                  |            | Mean | Minimum | Maximum | Negative samples | Positive samples |
| LG-food          | P-food     | 0.00 | 0.00    | 0.00    | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 12.75| 0.00    | 63.78   | 9 (90%)          | 1 (10%)          |
| FG-food          | P-food     | 0.00 | 0.00    | 0.00    | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 71.67| 0.00    | 358.36  | 9 (90%)          | 1 (10%)          |
| GFree-food       | P-food     | 0.00 | 0.00    | 0.00    | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 40.74| 0.00    | 203.72  | 9 (90%)          | 1 (10%)          |
| CG-food          | P-food     | 0.00 | 0.00    | 0.00    | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 41.19| 0.00    | 205.98  | 9 (90%)          | 1 (10%)          |

| Food ingredients | Food class | Mean | Minimum | Maximum | SD | SEM | P value |
|------------------|------------|------|---------|---------|----|-----|---------|
| LG-food          | Food ingredients | 71.45| 23.26   | 1.00    | 0.848 | 0.033 | 0.035 |
| FG-food          | Food ingredients | 0.00 | 0.00    | 0.00    | 0.848 | 0.033 | 0.035 |
| GFree-food       | Food ingredients | 0.00 | 0.00    | 0.00    | 0.848 | 0.033 | 0.035 |
| CG-food          | Food ingredients | 0.00 | 0.00    | 0.00    | 0.848 | 0.033 | 0.035 |
| Food class       | P-food     | 75.59| 0.00    | 377.96  | 18 (90%) | 2 (10%) |
|                  | E-food     | 968.57| 102.44  | 2267.78 | 16 (80%) | 4 (20%) |
| Total            |            | 36 (90%) | 4 (10%) |

LG-food: dog foods with lamb meal (or by products) + grain; FG-food: dog foods with fish meal (or by products) + grain; CG-food: dog foods with chicken meal (or by products) + grain; GFree-food: dog foods with animal by-products + grain-free; P-food : Premium foods; E-food : Economic (or with low price) foods.

### Table 3. Prevalence and concentrations of fumonisin-B1 in commercial dog foods with different ingredients.

| Food ingredients | Food class | Concentration, ppb in DM | Prevalence, % |
|------------------|------------|--------------------------|---------------|
|                  |            | Mean | Minimum | Maximum | Negative samples | Positive samples |
| LG-food          | P-food     | 75.59| 0.00    | 377.96  | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 968.57| 102.44  | 2267.78 | 9 (90%)          | 1 (10%)          |
| FG-food          | P-food     | 0.00 | 0.00    | 0.00    | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 923.51| 0.00    | 1527.89 | 9 (90%)          | 1 (10%)          |
| GFree-food       | P-food     | 0.00 | 0.00    | 0.00    | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 157.65| 0.00    | 446.51  | 9 (90%)          | 1 (10%)          |
| CG-food          | P-food     | 101.20| 0.00    | 506.02  | 9 (90%)          | 1 (10%)          |
|                  | E-food     | 1338.68| 0.00    | 3107.28 | 9 (90%)          | 1 (10%)          |

| Food ingredients | Food class | Mean | Minimum | Maximum | SD | SEM | P value |
|------------------|------------|------|---------|---------|----|-----|---------|
| LG-food          | Food ingredients | 752.68| 192.99  | 0.00    | 0.044 | 0.343 | 0.001 |
| FG-food          | Food ingredients | 0.00 | 0.00    | 0.00    | 0.044 | 0.343 | 0.001 |
| GFree-food       | Food ingredients | 0.00 | 0.00    | 0.00    | 0.044 | 0.343 | 0.001 |
| CG-food          | Food ingredients | 0.00 | 0.00    | 0.00    | 0.044 | 0.343 | 0.001 |
| Food class       | P-food     | 44.19| 0.00    | 506.02  | 18 (90%) | 2 (10%) |
|                  | E-food     | 847.10| 0.00    | 3107.28 | 18 (90%) | 2 (10%) |
| Total            |            | 24 (60%) | 16 (40%) |

LG-food: dog foods with lamb meal (or by products) + grain; FG-food: dog foods with fish meal (or by products) + grain; CG-food: dog foods with chicken meal (or by products) + grain; GFree-food: dog foods with animal by-products + grain-free; P-food : Premium foods; E-food : Economic (or with low price) foods.

The difference between the average values indicated by different letters at same column.

**Fumonisin-B1 in commercial dog foods**

According to the food ingredients, the fumanisin-B1 was detected in 60% of the studied LG-food. The fumonisin-B1 was detected in 40% of FG-food and 10% of premium dog foods and 70% of economic dog foods \( (P < 0.001) \). Fumonisin-B1 mycotoxin was detected in 40% of the total dog foods examined (Table 3).
The fumonisin-B1 concentration of premium class dog foods (44 ppb in DM) was lower than that (847 ppb in DM) of economic class dog foods (P < 0.001). The fumonisin-B1 concentration in GFree-food of dog (about 79 ppb in DM) was lower than those of other dog foods (P < 0.05). The highest fumonisin-B1 concentration of dog foods was in economic class of CG-food (3107 ppb in DM). The premium classes of LG-food and FG-food did not contain fumonisin-B1 mycotoxins (Table 3).

Zearalenone in commercial dog foods

The zearalenone was detected in 10% of CG-food, 20% of LG-food, and 30% of FG-food and GFree-food. The 35% of premium dog foods and 10% of economic dog foods included the zearalenone mycotoxin (P = 0.058) (Table 4).

The economic classes of LG-food and CG-food and the premium class of FG-food did not contain the zearalenone mycotoxin. The zearalenone concentration of GFree-food (about 6 ppb in DM) was lower than those (about 87, 108 and 118 ppb in DM) of LG-food, FG-food and CG-food (P < 0.05). Therefore, zearalenone concentration of premium class dog foods (about 153 ppb in DM) was higher than that of economic class dog foods (about 7 ppb in DM) (P < 0.05) (Table 4).

Total mycotoxin in commercial dog foods

The total mycotoxins (total aflatoxin + ochratoxin-A + fumonisin-B1 + zearalenone) concentration of GFree-food (106 ppb in DM) were lower than those of other dog foods (P < 0.01). The total mycotoxin concentration of economic class dog foods (897 ppb in DM) was higher than that of premium class dog foods (198 ppb in DM). The highest total mycotoxin concentration was in the economic class of CG-food (Table 5).

Discussion

Aflatoxins, ochratoxin-A, and Fusarium mycotoxins have been found in both raw ingredients and extruded products of pet food (Kepirska-Pacelik and Biel 2021a,b). Aflatoxin, a hepatotoxin and carcinogen, has caused several food poisoning outbreaks in dogs, and aflatoxin content is regulated in pet food in many countries. Ochratoxin A and Fusarium mycotoxins including trichotheeces, zearalenone, and fumonisins may have chronic effects on the health of companion animals (Leung et al. 2006). However, the actual prevalence of mycotoxins in and the status of economic or
premium class dog foods with cereal/grain or cereal-free/grain-free pet foods have been partially studied by a limited number of studies. In the present study, total aflatoxin (aflatoxin B1 + B2 + G1 and G2) has never been detected in the economic and premium classes of grain-free and fish+ grain-containing dog foods, and in general, premium-class foods contain a lower concentration of total aflatoxin. The median lethal dose (LD$_{50}$) of aflatoxin B$_1$ for the dog is of 0.5–1.5 mg/kg body weight; clinical manifestations are even observed at doses greater than 60 ppb of total aflatoxin in feed (Stenske et al. 2006). High doses of total aflatoxin are associated with acute forms of aflatoxicosis (Reis-Gomes et al. 2014; Arnot et al. 2012). In addition, studies of poisoning outbreaks in dogs have found very high AFB$_1$ values (<5.0 ppb) (Newman et al. 2007). In the present study, the concentration of total aflatoxin in economy class dog foods reached up to 6.85 ppb in DM, and it was seen that the risk of chronic aflatoxicosis was higher than premium class dog foods. The total aflatoxin concentration in economy class of CG-food containing chicken meat/by-products was higher than those of other dog foods proved that there was a risk of chronic aflatoxicosis in dogs. The total aflatoxin concentration of lamb and chicken foods with cereals were higher than those of dog foods containing fish meat that aflatoxin contamination was present in these meat sources. Cereal products, which constitute an important part of the diets of farm animals (lamb or chicken), may cause aflatoxin residues in lamb and chicken meat with total aflatoxin presence, which determined in the present study.

Ochratoxins are a group of mycotoxins produced by some Aspergillus species (mainly A. ochraceus and A. carbonarius, but also by 33% of A. niger industrial strains) and some Penicillium species, especially P. verrucosum. Ochratoxin-A is the most prevalent and relevant fungal toxin of this group, while ochratoxin-B and C are of lesser importance. In the cuttent study, ochratoxin-A has not been detected in any of the premium class foods and its prevalence is low (4% for 40 dog food brand). The value in which ochratoxin-A was detected at the maximum concentration (358 ppb in DM) was in the foods containing grain+ fish. Ochratoxin-A and total aflatoxin were present in dry type dog foods at a lower concentration than the other mycotoxins studied. Tegzes et al. (2019) stated that ‘none of the samples analyzed contained detectable concentrations of aflatoxin, demonstrating how effective regulatory and control strategies were in reducing the incidence of aflatoxin in dry commercial dog foods.’ In the presented study, it was determined that the mycotoxins produced by Fusarium fungus were the highest rate of mycotoxins, and this result was similar to the previous study data (Bissoqui et al. 2016). In a study conducted in Austria, fumonisin and zearalenone mycotoxins were detected in dog foods (Böhm et al. 2010). A study conducted in Brazil reported low levels of most mycotoxins in dry dog foods (Bissoqui et al. 2016).

The highest concentrations of fumonisins-B1 and zearalenone in dog foods in the present study, were 3107 ppb (in economic class CG-food) and 864 ppb (in premium class LG-food), respectively. The maximum fumonisin-B1 concentration of Gfree-food was 446 ppb, and this Fusarium mycotoxin of Gfree-food was lower than those of dog foods with cereal ingredients. The maximum concentrations of fumonisin-B1 of the researched premium and economic dog foods were 506 and 3107 ppb in DM. Previous a study showed that the highest concentrations of fumonisins-B$_1$ and zearalenone were 74.83 and 45.84 ng/g, respectively (Witaszak et al. 2019). Witaszak et al. (2020) stated that mycotoxins in both cat and dog food samples from Polish market were detected in the amounts ranging from 0.3–30.3, and 12.3–53.0 ng/g for zearalenone, and fumonisin-B$_1$, respectively. In the

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Table 5. Concentration of total mycotoxin in commercial dog foods with different ingredients.

| Food ingredients | Food class | Mean | Minimum | Maximum |
|------------------|------------|------|---------|---------|
| LG-food P-food   | 291.65     | 0.00 | 1244.02 |
| LG-food E-food   | 982.27     | 167.42 | 2269.15 |
| FG-food P-food   | 160.76     | 0.62 | 663.75  |
| FG-food E-food   | 1012.75    | 0.84 | 1655.15 |
| GFree-food P-food| 1.60       | 0.56 | 3.02    |
| E-food           | 211.94     | 0.00 | 548.66  |
| CG-food P-food   | 338.91     | 1.60 | 805.56  |
| E-food           | 1382.23    | 1.29 | 3318.30 |

**Food ingredients**
- LG-food: dog foods with lamb meal (or by products) + grain
- FG-food: dog foods with fish meal (or by products) + grain
- GFree-food: dog foods with animal by-products + grain-free
- P-food: Premium foods
- E-food: Economic (or with low price) foods

**Food class**
- LG-food: dog foods with lamb meal (or by products) + grain
- FG-food: dog foods with fish meal (or by products) + grain
- GFree-food: dog foods with animal by-products + grain-free
- P-food: Premium foods
- E-food: Economic (or with low price) foods

**Total mycotoxin, ppb in DM**

| SD    | 779.89 |
| SEM   | 215.52 |
| P value | 0.011 |
| Food ingredients | 0.003 |
| Food class | 0.569 |
| Food class * | 0.369 |

**Table 5.** Concentration of total mycotoxin in commercial dog foods with different ingredients.
current study, in terms of fumonisin-B1 mycotoxin, premium class dog foods and grain-free dog foods were determined to have lower content. However, none of the first-class grain-free dog food and the first-class fish + cereal food contained fumonisin-B1. Another study (Tegzes et al. 2019) found Fusarium mycotoxin, produced by Fusarium fungus, induced in grain-containing dry dog food, but no Fusarium mycotoxin was found in grain-free dry-type dog food or wet dog food. In the present study, it was determined that the level of fumonisin-B1 was approximately 6 times higher in economy class dog foods compared to premium class dog foods, and this mycotoxin level was at a higher concentration than the others. Similarly, in previous researchers (Macías-Montes et al. 2020) it was determined that fumonisins (B1 and B2) were the highest mycotoxin found in dry type foods (especially cat foods) of dogs and cats. In a recent study, it was determined that there was almost no difference in the level of contamination between expensive and inexpensive brands of pet food, some brands had alarmingly high fumonisin levels, but a low risk of acute toxicity, and a moderate chronic risk for zearalenone and fumonisins (Macías-Montes et al. 2020). The highest concentrations of zearalenone mycotoxin in the studied dog foods were in LG-food as 3318 ppb and economic class (mean 211 ppb, minimum-maximum 0.56–3.02 ppb) and economic class (mean 211 ppb, minimum-maximum 0.0–548 ppb) of GFree-foods.

The concentration and prevalence of ochratoxin-A and total aflatoxin in the studied dog foods were lower than those of fumonisin-B1 and zearalenone may indicate that extruded processes or other processes inhibited the production of toxins by fungi. In general, the fact that premium dog foods are lower in terms of total aflatoxin, fumonisin-B1 and ochratoxin-A concentration and prevalence compared to economic dog foods is due to the content of premium dog foods or the processes applied. As expected, grain-free dog foods did not include aflatoxins because they do not contain cereal grains that are important aflatoxins contaminants. However, it has been observed that some mycotoxins (ochratoxin-A, fumonisin-B1 and zearalenone) are contaminated in grain-free dog foods (especially economic class).

In the present study, the zearalenone, a Fusarium mycotoxin, was detected in 10% of CG-food, 20% of LG-food, and 30% of FG-food and GFree-food. In addition, the 35% of premium dog foods and 10% of economic dog foods included the zearalenone mycotoxin. Witaszak et al. (2019) demonstrated that only 9.5% of the forty-two samples of veterinary diets for dogs and cats were free from Fusarium mycotoxins (fumonisin-B1, deoxynivalenol, nivalenol and zearalenone). In the present study, the fumonisin-B1 was detected in 40% of FG-food and GFree-food. The fumonisin-B1 was detected in 10% of premium dog foods and 70% of economic dog foods. Fumonisin-B1 mycotoxin was detected in 40% of the total dog foods examined. It is thought that the differences in the prevalence of Fusarium mycotoxins in the studies will depend on the number of commercial foods used in the study, the economic and premium foods, the raw material content of the foods, storage conditions and shelf life. A study conducted in Poland found multiple mycotoxins in dry type dog foods (Witaszak et al. 2019). Among the various mycotoxins detected in pet foods, zearalenone was detected in 69% of the samples, deoxynivalenol in 52%, fumonisin-B1 in 33% and nivalenol in 26% (Witaszak et al. 2019).

Grain processing, sampling error, analytical methods, conjugated mycotoxins, storage conditions and synergistic interactions are common challenges faced by the pet food industry. Food processing techniques such as screening, washing, polishing, ozonation and acid-based mould prevention reduce the mycotoxin content of cereal grains. Dietary supplementation with major neutral amino acids, antioxidants and omega-3 polyunsaturated fatty acids, and the inclusion of mycotoxin sequestering agents and detoxifying microorganisms can ameliorate the deleterious effects of mycotoxins in contaminated pet foods (Leung et al. 2006). Raw grains, food ingredients and finished food are subject to certain regulatory guidelines (FDA 2001; Marin et al. 2013), but mycotoxin contamination is particularly difficult to prevent because mycotoxins are relatively resistant to heat and chemical inactivation processes in downstream processing steps (Bissoqui et al. 2016; Marin et al. 2013). The high concentration/prevalence of ochratoxin-A and fumonisin-B1 in the cereal-based dog foods (LG-food, FG-food and CG-food) from economy-class may be due to the low price of the cereal flour and by-products (rice and other cereals), which were the increased shelf life and do not have ideal storage conditions. However, the high concentration or prevalence of the high prevalence of fumonisin-B1 and ochratoxin-A in the grain-free dog foods from E-food class examined in the present study had that it may also be caused by meat meal or meat by-products, which are other raw materials other than cereal grain (Montanha et al. 2017). High levels of ochratoxin-A or fumonisin-B1 in meat...
and meat products have been attributed to the feeding of animals with ochratoxin-A-contaminated feeds or the contamination of meat products with fungi (Tolosa et al. 2021). Fumonisins-B1, which are characterised by low oral absorption and rapid plasma elimination, is not considered to accumulate in animals (Howard et al. 2001). However, recent studies in chicken and turkey showed that, in these species, the hepatic half-elimination time of fumonisins-B1 was several days, suggesting that FB1 may accumulate in the body (Laurain et al. 2021). Laurain et al. (2021) found that fumonisins-B1 could accumulate at higher levels in chicken tissues with an increase in the time of exposure and in the age of the animals. In the presented study, the high concentration of fumonisins-B1 mycotoxin in dog foods containing chicken meal or by-products may be due to the possibility of accumulation of this mycotoxin in chicken tissues (Laurain et al. 2021). In the animals, it has been stated that zearalenone or its metabolites can pass to animal products (meat, egg, milk) in animals fed with feed containing zearalenone, but this is very limited in normal production systems. Interestingly, the high prevalence and concentration of zearalenone in P-foods in the present study had according to these of E-foods may be related to the possible raw materials (animal protein source or cereals) in their content (Laurain et al. 2021; Singh et al. 2018). However, it may not be realistic to completely attribute the difference in mycotoxin content of dog foods with different raw material contents to the food ingredients. The possibility of this effect on cooling, packaging and storage conditions, which are the stages after the extruded process of the dog food, should not be overlooked (Pęsi et al. 2014).

Conclusion
As a result, grain-free dog foods contain very low concentrations of fumonisins-B1 and zearalenone mycotoxins, while premium dog foods contain none. Premium class foods contain Fumonisins-B1 at lower concentrations than economic classes. Ochratoxin-A has not been detected in any of the premium class foods and its prevalence is low. Interestingly, economy-class foods contain zearalenone mycotoxin at a lower concentration than premium-class dog foods. Total aflatoxin has never been detected in the economic and premium classes of grain-free and fish + grain containing foods, and in general, premium-class foods contain a lower concentration of total aflatoxin.

Acknowledgments
The author thanks the Research Fund of Erciyes University (Kayseri, Turkey).

Ethics approval
The author confirms that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards to protect animals for scientific purposes and feed legislation.

Consent for publication
The author has consented to the publication and presentation of the data in this article.

Author contributions
Conceptualisation: Kanber KARA; Methodology: Kanber KARA; Formal analysis and investigation: Kanber KARA; Writing - original draft preparation: Kanber KARA; Writing - review and editing: Kanber KARA; Resources: Kanber KARA; Supervision: Kanber KARA.

Disclosure statement
The author declares the she has no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding
This study was supported by the Research Fund of Erciyes University (Kayseri, Turkey).

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Data availability statement
The data analysed in this investigation are available upon request to the corresponding author.

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