

Fungi and aflatoxin B₁ in horse and dog feeds in Western Turkey

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SUMMARY

To evaluate fungi growth and aflatoxin B₁ contamination in horse and dog feeds, total of 41 feed samples were examined with agar plate incubation and ELISA methods. Fungi growth of the horse feed was not exceeding maximum tolerable limits (0.97×10^4 CFU/g). Although dog foods should not contain any fungi contamination, slightly less than half of the tested dog foods had fungi growth. Both feeds had aflatoxin B₁ content in allowable limits (10 µg/kg.), however, dog foods contain significantly higher aflatoxin B₁ content than horse feeds do ($P \leq 0.05$) (6.69 µg/kg and 1.98 µg/kg, respectively). Fungi growth and aflatoxin B₁ content did not correlate in either group of feeds ($r^2 = 0.09$ and $r^2 = 0.30$, respectively). Surveillance of both fungi growth and aflatoxin contents of both types of feeds are necessary for the health of the animals.

KEY-WORDS : horse - dog - feed - fungi - aflatoxin B₁.

RÉSUMÉ

Les moisissures et l'aflatoxine B₁ dans la nourriture du cheval et du chien la Turquie occidentale. Par M. BASALAN HISMIOGULLARI, E. SAHVER HISMIOGULLARI, A. ADNAN et A. FILAZI.

Pour évaluer le développement de moisissures et la contamination par de l'aflatoxine B₁ dans la nourriture pour cheval et pour chien, 41 échantillons d'aliments ont été examinés par incubation sur agar et par dosage mycotoxique selon la méthode ELISA. Le développement de moisissure dans la nourriture de cheval n'a pas dépassé les limites tolérables (0.97×10^4 CFU/g). Bien qu'on ne désire pas de moisissure dans les aliments pour chien, on a constaté la présence de contaminants dans presque la moitié des échantillons analysés. Dans les deux types d'aliments, le taux d'aflatoxine B₁ se trouve dans des limites tolérables (10 µg/kg.). Toutefois, nous montrons que la teneur en aflatoxine B₁ est statistiquement plus élevée dans la nourriture pour chien que dans celle destinée aux chevaux ($P \leq 0.05$) (6.69 µg/kg et 1.98 µg/kg, respectivement). Nous n'avons pas mis en évidence de rapport direct en développement fongique et teneur en aflatoxine et ce dans les deux types d'aliments testés ($r^2 = 0.09$ et $r^2 = 0.30$ respectivement). Toutefois, la surveillance de la contamination fongique et celle de la teneur en aflatoxine B₁ dans les aliments pour animaux est nécessaire afin d'assurer la salubrité de ces produits.

MOTS-CLÉS : cheval - chien - nourriture - moisissures - aflatoxine B₁.

Introduction

Agricultural crops can become contaminated by fungi during production, storage, processing and transportation when temperature and humidity conditions are suitable. Because fungi and their metabolites (mycotoxins) can present a major risk to human and animal health, producers seek to prevent fungal growth and avoid mycotoxins.

Hundreds of fungi species of more than twelve genera produce more than three hundreds identified mycotoxins which can pose a threat to health of all mammalian species [6, 7, 10, 22]. Besides temperature and moisture, other environmental

factors such as plant composition and texture, plant stress and drought and insect damage allow mycotoxin-producing fungi to penetrate the skin of crops or grains [15]. One survey indicated that 25 % of global agricultural commodities is contaminated with mycotoxins [5, 7]. Mycotoxicosis caused by chronic exposure to low doses are seen more frequently than that by acute exposure to high doses ; acute and sub-acute intoxication occasionally occur when animals are exposed to high concentrations of mycotoxins such as aflatoxins (B₁, B₂, G₁, and G₂), Ochratoxin A, fusarium species (deoxynivalenol, nivalenol, T-2 toxin, zearalenone and fumonisin B₁) for even a short period of time [3, 6, 7, 13, 14].

Health problems associated with aflatoxin ingestion include immuno-suppression, mutagenity, teratogenity, cancer and liver damage [3, 13, 14, 18, 22]. Since the clinical signs of acute aflatoxicosis in affected livestock are not specific for aflatoxicosis [21], many field outbreaks go undiagnosed. Acute aflatoxicosis in dogs first defined as hepatitis X [13], later close resemblance of its symptoms to «moldy corn poisoning» in swine led the researchers to acute aflatoxin toxicosis [14]. However, the impact of chronic aflatoxicosis may even seem more drastic due to reduced productivity and increased susceptibility to infections of livestock [5].

The two animal species most sensitive to aflatoxicosis are rainbow trout and ducklings [5]. Although most organs are affected by acute or chronic exposure, liver is the primary organ affected with severe aflatoxicosis causing haemorrhagic necrosis, fatty infiltration and bile duct proliferation [3, 5, 13, 14]. Kidney lesions were detected in pigs fed high doses of aflatoxin B₁ and ochratoxin A [21]. With cattle, ruminal motility (contraction frequency and amplitude) was altered by high doses of aflatoxin [4].

Poultry, swine and cattle feeds have been screened extensively for mycotoxins [7, 22]. Since mycotoxin production by specific fungi on agricultural commodities occur during storage, October to April was the predominant season for detection of aflatoxin B₁ in dog feed [13] in South Africa [3], and poultry feed in Argentina [6]. Among the individual feed-stuffs analysed for aflatoxin B₁ and aflatoxin B₂, ground corn and cottonseed cake had the highest aflatoxin concentrations. Fish meal was contaminated by aflatoxin B₁ and aflatoxin B₂ more frequently than wheat bran and soyabean meal [12]. Maize and grain by-product samples contained a very wide range in concentrations of aflatoxin B₁, B₂, G₁ and G₂ in Indonesia [17] presumably due to the tropical conditions ideal for fungal growth. ABARCA and his co-workers [1] pointed out that pelleted feeds for rabbits in Spain contained very low concentrations of mycotoxigenic fungi because of the heat and steam treatment associated with pelleting.

Few research studies have focused on mycotoxins in horse and pet foods possibly because productivity of such animals are rarely of interest. This study was designed to examine fungi presence and aflatoxin B₁ concentrations in horse and dog feeds and to determine the correlation between fungi growth and aflatoxin B₁ concentration in both feeds.

Material and methods

PREPARATION OF SAMPLES

Twenty samples of pelleted horse feeds and 21 samples of pelleted dog foods were collected from 19 different market-places including retail stores, factory outlets and contract dealers and 7 different manufacturing plants located in the Western region of Turkey. Samples were taken from storage immediately after being in 50 kg nylon bags. Samples collected from several bags were mixed together and held at 4°C for fungal and mycotoxin analysis the day after collection. Samples were kept in polyethylene bags until fungi counts were determined within 7 days of collection.

FUNGI COUNTS

Rose-bengal chloramphenicol agar (16 g) (Oxoid Co., Hampshire, UK) was suspended in 500 ml of distilled water ; the mixture was well agitated and boiled to fully dissolve the agar. Then, contents of one vial of chloramphenicol selective supplement SR78 containing 0,05 g chloramphenicol were added. This was sterilised by autoclaving (Nuve San., Ankara, Turkey) at 121°C for 15 minutes and allowed to cool to 50°C. The media was mixed well and poured into sterile petri dishes (Sigma-Aldrich Chemie GmbH, Diesenhofen, Germany).

For culturing, 10 g of ground feed sample was added to 90 ml of 0.1 % peptone water, allowed to stand for 15 minutes, and shaken in a Seward stomacher (Cole parmer Co., Illinois, USA) for 15 minutes. Samples were collected randomly from various places of the stomacher. From this diluted sample, 0.1 ml was pipetted onto the surface of petri plates containing the rose bengal chloramphenicol agar base. The petri plates were incubated at 25°C for 5 to 7 days. All colonies without identification of specific fungi types were counted and multiplied by the dilution factor (100) to calculate Colony Forming Unit (CFU) for per g of animal feed. Duplicate samples from each feed culture were used to check repeatability of the sampling and incubation procedure.

AFLATOXIN B₁ ANALYSIS

Methanol, n-heptane, dichloromethane and PBS-buffer (Sigma-Aldrich Chemie GmbH, Diesenhofen, Germany), pH 7.2 (0,55 g NaH₂PO₄ x H₂O ; 2,85 g Na₂HPO₄ x 2H₂O + 9 g NaCl diluted to 1000 ml with distilled water) were used. A finely ground sample (2 g) of each animal feed was weighed into a screw-top glass vial ; 10 ml of methanol / distilled water (70 / 30) were added to each glass tube and tubes were shaken for 15 minutes. The suspension was filtered through paper filter (Whatman # 3) and 100 ml of the filtered suspension was transferred to a clean glass tube and 600 ml of the sample dilution buffer was added. A sample of the final suspension (50 ul) was pipetted into each well for the assay (Nubenco Inc., New Jersey, USA). Aflatoxin B₁ (Catalog number A6636, Sigma-Aldrich Chemie GmbH, Diesenhofen, Germany) was used as a standard. The aflatoxin B₁ concentration was calculated in µg/kg (ppb).

STATISTICAL ANALYSIS

For statistical analysis, the GLM procedure of SAS [19] was used. Mean fungi growth and aflatoxin B₁ contents of the two different types of feed were compared by LSD. The correlation between fungi growth and aflatoxin B₁ content was calculated within each feed type with a difference of P ≤ 0.05 being considered to be statistically significant.

Results and discussion

Main ingredients and nutrient contents of the horse feeds and dog foods as listed on the feed tag label are listed in Table I. Both feed types were dry and pelleted but ingredients ori-

Nutrient	Dog Food ¹	Horse Feed ²
Crude protein	26	15
Crude fat	18	na
Crude fibre	4,5	11
Moisture	10	12
Crude ash	na	8
Ca	na	1
P	na	0,6
Metabolizable Energy	na	2400

¹ dog food contains chicken by-product meal, ground yellow maize, meat meal, ground wheat, chicken fat, (preserved with mixed tocopherols, a source of natural vitamin E), dried beet pulp, fish meal, dried whey products, salt, vit. A supplement, vit. D₃, vit. E, riboflavin, D-calcium pantothenate, niacin, choline chloride, vit. B₁₂, folic acid, thiamine mononitrate, pyridoxine hydrochloride, biotin, ethylenediamine dihydroiodide, copper sulfate, ferrous sulfate, manganous oxide, zinc oxide, magnesium oxide,

² horse feed contains grains and grain by-products, oil-seed meals, fats, starch by-products, limestone, dicalcium phosphate, salt, sodium bicarbonate, molasses, vitamin and mineral premixes
na = not available

TABLE I. — Guaranteed nutrient concentrations in pelleted feeds for horses and dogs, % DM.

generated from different sources. Feedstuffs in the horse feeds were mainly agricultural crops and by-products whereas components of dog foods came from animal sources. This difference in ingredient sources may be responsible for the greater fungi growth and aflatoxin concentrations of dog food than horse feed.

The Agricultural Ministry of Turkey set the maximum allowable limits for fungi and aflatoxins in food sources and animal feeds in 1990 [2]. According to this standard, fungi contamination of the horse feed should not exceed 5.0×10^4 CFU/g, while dog food should not have any contamination with fungi. Mean fungi content of the horse feed was $0.97 \times 10^4 \pm 0.24 \times 10^4$ CFU/g so the average was lower than maximum tolerable limit and none of the 20 tested samples exceeded the maximum allowable limit (Figure 1). In contrast, nearly half of the tested dog food (10 out of 21) were contaminated with fungi and therefore would be considered unsafe for dogs to consume (Table II). Although the prevalence of individual fungal types was not identified, the presence of fungi suggests that these feeds may present a health hazard for animals.

Aflatoxin B₁ content of horse feed averaged 1.98 (+/- 0.71) while dog food averaged 6.69 (+/- 1.65) µg/kg. The maximum allowable limit for aflatoxin B₁ in mixed feeds for horses and dogs were set has been set at 10 µg/kg [5]. Dog feed contained more ($p \leq 0.05$) aflatoxin B₁ than horse feed, but neither horse feeds nor dog foods exceeded the legal limit.

In contrast to common assumptions, fungi growth and aflatoxin B₁ did not correlate in either feed type ($r^2 = 0.09$ and $r^2 = 0.30$ for the horse feeds and dog food respectively) (Figure 2). This result supports the observation of FINK-GREMMELS [7] who cited that a feed might contain aflatoxin B₁ even though it shows no fungal activity. Feeds previously contaminated with fungi might have aflatoxins but growth and survival of fungi may have been halted by fungicides or other means that kill fungi and fungal spores but do not destroy the mycotoxins.

Since horses are fed for their performance, low concentrations of fungi in feed might present a danger if the feed is stored over a large period of time under unfavorable conditions even though the fresh feed does not contain an amount of mycotoxin considered to be dangerous [6]. Effective surveillance of agricultural commodities during storage and transportation for prevention of fungi growth and mycotoxin contamination is needed. Presence of Aflatoxin B₁ in horse feed indicates that improper storage conditions may have occurred prior to pelleting as heat treatment during pelleting would have destroyed the fungi but would not affect Aflatoxin B₁. Samples collected from marketplaces might have been re-contaminated with fungi which produce aflatoxin B₁.

Although the level of Aflatoxin B₁ in feed ingredients in European community is set at 10 µg/kg, Netherlands has decreased this limit to 5 µg/kg [1]. If that ceiling were used, the mean Aflatoxin B₁ concentration for the horse feeds would have exceeded that limit.

EMBED

Fungi Growth	Aflatoxin B ₁ concentration (mg/kg) ¹										
	0.45	0.015	2.7	0.1	0.3	0.58	20	9	9.5	4	3.5
-											
+	3.4	2	4.5	2.2	3	3.5	24	23	15	9	

¹ Correlation coefficients (r) = 0.30 between fungi and aflatoxin B₁

TABLE II. — Fungi growth and aflatoxin B₁ concentrations of dog feeds.

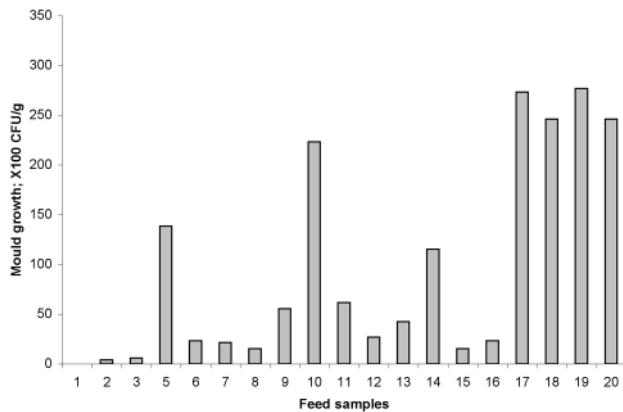


FIGURE 1. — Fungi growth (X10² CFU/g) of the horse feed samples (n = 20).

Identification of specific aflatoxigenic strains of fungi would have increased the correlation between fungi count and aflatoxin concentration [1, 20]. A high fungi count (CFU/g) with low Aflatoxin B₁ concentration could be attributed to presence of non-aflatoxigenic strains of fungi or suitable environmental conditions such as humidity, temperature and substrates. The opposite could be explained by contamination of feeds prior to stabilizing the feed or pelleting.

Since most horses are used for racing and other types of competition, it is hard to detect any adverse effects of feeding mouldy and mycotoxin-containing feeds unless clinical signs occur whereas performance depressions are readily detected with dairy cattle or poultry. However, serious health consequences beside low performance including respiratory infections and decreased immune responsiveness still might cause major economic loss so extraordinary attention should be paid for fungi infestation of horse feeds.

A similar scenario exists for dogs and their companionship with humans. In addition, several studies indicate that pet foods are consumed by humans because pet foods usually are economical, nutritionally balanced, and tasty. Based on such studies, health standards for pet foods should equal those of human foods. Concern for pets has resulted in stricter regulations for pet foods than feeds for production livestock. These regulations must be enforced. Diseases caused by aflatoxin B₁ are usually irreversible and severe ; characterized with neural, liver, and kidney damages, so surveillance of dog food is life-worthy. Another area that requires special attention is that dog diets are rich in protein and ingredients are derived from animals. Spoilage or fungi infestation of such ingredients might cause complex illnesses that are difficult or impossible to diagnose [21].

Contamination of the plant-animal-human food chain is difficult to control and must involve in various levels [16, 17]. Although quality control is difficult, preventing contamination is more fruitful than treating the many-fold health consequences of fungi [9, 22]. Risk areas for contamination should be identified correctly and fungal growth must be

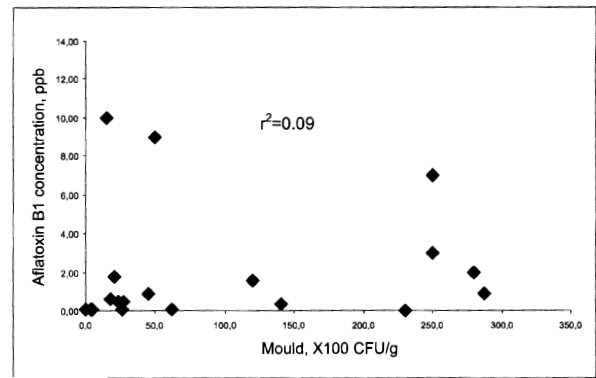


FIGURE 2. — Fungi growth (X10² CFU/g) and aflatoxin B₁ concentration of the horse feed samples (r² = 0.09).

reduced by proper storage and handling of foods and feed and by producing high quality pellets [8] or adding chemicals [22] including phosphate [11] or ammonia treatment or adding adsorbants such as silica clay, or enzymes such as epoxidase and esterase [7].

In conclusion, horse feeds and dog foods should be inspected routinely for fungal growth. Potential for aflatoxin production also should be detected by valid testing as well as fungi identification procedures and tools. Considering the health hazards of mycotoxins, it is critically important to monitor animal foods for presence of mycotoxins and fungi.

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