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RESEARCH ARTICLE

Analysis of nutritional contents, microbiological contamination and presence of aflatoxins in unpacked feeds sold for pet-birds in Istanbul

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İstanbul'da paketsiz satılan evcil kuş yemlerinin besin içeriklerinin, mikrobiyolojik kontaminasyonun ve aflatoksin varlığının analizi

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Öz

Amaç: Bu çalışmada İstanbul'da satılan paketsiz evcil kuş yemlerinin besleyici yeterliliği, mikrobiyolojik kalitesi ve aflatoksin içeriğinin tespit edilmesi amaçlanmıştır.

Gereç ve Yöntem: İstanbul'un farklı bölgelerinden toplam 102 adet paketsiz yem örneği (35 kanarya, 37 muhabbet kuşu, 30 papağan) toplanmıştır. Tohum karışımlarından oluşan tüm örneklerin besin madde içeriği, mikrobiyolojik kalitesi ve aflatoksin içeriği analiz edilmiştir.

Bulgular: Muhabbet kuşu yemlerinin ham protein (HP), ham yağ (HY) ve metabolize olabilir enerji (ME) içeriği ihtiyacı karşılayabilecek düzeydedir. Ancak kanarya ve papağan yem örneklerinin HP, HY ve ME düzeyinin ihtiyaçtan daha yüksek düzeydedir. Kanarya ve muhabbet kuşu yemlerinin yaklaşık %40'ında (sırasıyla 3.19 ± 0.16 ve 3.25 ± 0.11 log cfu/g) , papağan yemlerinin ise tamamında (3.43±0.58 log cfu/g) koliform bakteri izole edilmiştir. Yem örneklerinin tamamında 3-5.5 log cfu/g oranında küf tespit edilmiştir. Salmonella izole edilememiştir. Aflatoksin B1, B2, G1 ve G2 hiçbir yem örneğinde bulunmamıştır.

Öneri: Mikrobiyolojik sonuçlar evcil kuşlar üzerinde tehtid oluşturmazken, kanarya ve papağan yemlerinin dengesiz besin madde içeriğinin evcil kuşların sağlıklarını olumsuz etkileyebilecek düzeyde olduğu anlaşılmıştır.

Anahtar kelimeler: Besin maddeleri, tohum karışımı, evcilkuş, yem hijyeni, Koliform

Abstract

Aim: In this study, it was aim to determine the nutritional efficiency, microbiological quality and aflatoxin contents of unpacked pet-bird feeds sold in Istanbul.

Materials and Methods: A total of 102 unpacked feed samples sold for canary (n=35), budgerigar (n=37) and parrot (n=30) were collected from different districts in Istanbul. All the samples, consist of seed mixtures, were analysed regard to their nutritional content, microbiological quality and presence of aflatoxins.

Results: Crude protein (CP), crude fat (CF) and metabolizable energy (ME) levels of the budgerigar feeds were met the nutritional requirements. However, nutritional contents (CP, CF, ME) of the canary and parrot feeds were higher than their requirements. In the microbiological analysis, *Coliform* group of bacteria were isolated from ~40% of budgerigar and canary feeds (3.19 ± 0.16 and 3.25 ± 0.11 log cfu/g, respectively), while isolated from all of the parrot feeds (100%; mean 3.43±0.58 log cfu/g). None of the 102 feeds were contaminated with *Salmonella*. Mould content of the feeds (100%) was ranging from 3 to 5.5 log cfu/g. Aflatoxin content (B1, B2, G1, G2) was not detected in feed samples.

Conclusion: Regarding to the microbiological results of seed mixtures no threat is found for pet birds. However, it is assumed that nutritional imbalance formulation of canary and parrot feeds may affect adversely the pet birds' health.

Keywords: nutrients, seed mixture, pet-bird, feed hygiene, *Coliform*

Introduction

Pet birds are commonly fed with seed mixture based on barley, canary seeds, oats, rice, sunflower, rapeseed, and varies of millet. But these seed mixtures have some disadvantages due to poor quality and imbalance formulation. Researches recently doubt whether seed mixtures can meet the nutritional requirements of pet birds. Most of the studies about the nutrition of pet birds compare the ingredients of seed mixtures with the nutritional requirements of domestic farm poultries. However, exotic birds have different nutritional habits and metabolism from others' (Ullrey et al 1991, Brightsmith 2012). In addition, because of economic reasons and easy accessibility, pet bird owners prefer unlabeled and unpacked seed mixtures to well-formulated feed for their birds without supplementary feedstuff lifelong. Thus, the imbalance of nutrient contents (such as fat, protein, energy) of seed mixtures cause both some metabolic diseases and decreasing longevity of pet bird (Bauck 1995, Bavelaar and Beynen 2004).

In addition to the nutritional content, feed hygiene is also essential for the birds' health. Harmful bacteria such as *Salmonella* or toxin producer moulds might occur in animal feeds during storage (FAO 2004). *Coliform* itself doesn't cause illnesses in pet birds but indicates a low feed hygiene related to the fecal contamination in feed (Maciorowski et al 2007). *Salmonella* is a well-known pathogen which might cause diarrhea and decrease immunity of avian. Toxin producing moulds have hepatotoxic effects and accordingly might pose a health risk of pet bird (Boermans and Leungs 2007, Mamun et al 2011). Feed contaminated with these microorganisms or their toxins may decrease feed intake and accordingly leads to malnutrition of pet bird.

Although the level and the frequency of contaminants and nutritional ingredients differ between studies, most of them were focused on the packed feeds. However unpacked seed mixtures are empirical formulated and more susceptible to contamination (Aquino and Correa 2011). Therefore the purpose of this study was to investigate the nutritional value, hygienic quality and mycotoxin content of pet bird seed mixtures sold unpacked in retail markets in Istanbul.

Materials and Methods

Sample collection

Totally one hundred and two samples of seed-mixes (35 for canary, 37 for budgerigar, 30 for parrot) were collected from several districts of Istanbul where the unpackaged feeds were sold by the retailers. Quadruple samples (0.5 kg each) were collected in a sterile polyethylene bag from different sides of the 25 kg seed mixture lots, and homogenized aseptically. The samples were transported to the laboratory within 2 hours for microbiological analysis. Remaining samples were stored at 4°C for nutritional and toxicological analysis on the following day.

Nutritional analysis

A half of kilogram from each samples was mechanically dehulled by the abrasive dehuller with an air aspirator to remove the hulls. Then, kernel samples were well grinded by a water cooled miller (M20, IKA). Crude protein (CP, method 988.01), crude fat (CF, method 920.39), crude fibre (method 962.09), moisture (method 930.15) and ash (method 942.05) contents of kernel samples were measured in accordance with the methods of AOAC (AOAC 1990). Soluble carbohydrate (nitrogen free extract, NFE) contents were calculated by subtracting the percent CP, CF, crude fibre, moisture and ash from 100 (Werquin et al 2005):

Soluble carbohydrate (NFE, %) = 100 – (CP + CF + Crude fibre + Moisture + Ash)
The metabolizable energy (ME) was calculated according to the formula (Werquin et al 2005):
ME (kj/g) = [(CP × 18) + (Soluble carbohydrate x 17) + (CF × 39)] / 100

Results of nutritional analyses were demonstrated in figures with the comparison of the nutritional requirements given in selected reference studies. The reference values of CP, CF and metabolizable energy were respectively considered as 14%, 4% and 14.66 kj/g for maintenance of adult canary and 12%, 4% and 13.40 kj/g for maintenance of adult budgerigar and parrot (Harper and Turner 2000, Koutsos et al 2001a, Orosz 2014).

Microbiological analyses

All of the feed samples were weight 25 g in sterile stomacher bag. 225 ml enrichment broth (Buffered Peptone Water, OXOID CM0509) was added into sample and incubated at 37°C for 24 h for Salmonella growth (Omurtag et al 2012). For the Coliform and mould isolation, the samples were diluted (1:10) in peptone water (OXOID, CM0009). Aliquots were taken to tenfold serial dilutions and streak onto the coli ID medium (Biomerieux 42017) for Coliform (incubated at 37°C for 24 h) (Omurtag et al 2012) and Dichloran Glycerol (DG-18) Agar (OXOID CM0729) for total mould count (incubated at 25°C for 5–7 days) (Copetti et al 2009). The enriched aliquots for Salmonella isolation were inoculated onto the MSRV motility agar (OXOID CM0910) and incubated at 42°C for 24 h. Salmonella suspect samples were streaked onto XLD-agar (Merck Nr.1.05287) and incubated at 37°C for 24 h.

Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) analyses

Standards, chemicals and instruments

Aflatoxin detection was performed with Agilent 1100 series HPLC Value System (USA), standarts and EASIE-EXTRACT® AFLATOXIN Immunoaffinity columns from R-Biopharm AG (Darmstadt, Germany), solvents from Merck (Darmstadt, Germany), ODS columns (5 µm, 4.6 mm x 150 mm,) from Hicrom Ltd. (UK) and Dionex UltiMate 3000 FLD as fluorescence detector from Thermo Fisher Scientific Inc. (MA, USA).

Preparation of solutions and calibration for HPLC

For the preparation of AF mix (Grade II) stock solution, 1 ml main stock solution was added to 10 ml toluene/acetonitrile (98:2 v/v). Concentrations of Grade II stock solution were 1000 ng/ml for AFB1-G1 and 200 ng/ml for AFB2-G2. For the preparation of Grade III stock solution, all Grade II stock solution was completed to 10 ml with toluene/acetonitrile (98:2 v/v). After mixing, concentrations of Grade III were 0.1 ng/ml for AFB1-G1 and 0.02 ng/ml for AFB2-G2. 10, 30, 50, 70 and 90 µl of Grade III stock solution were added into vials and it was kept under stream nitrogen at ambient temperature to evaporate toluene/acetonitrile solution. To resolve aflatoxins, 1 ml of HPLC grade methanol was added to each vial and shaken well. This mixture was completed to 2.5 ml with ultra-pure water and assayed to calibrate HPLC.

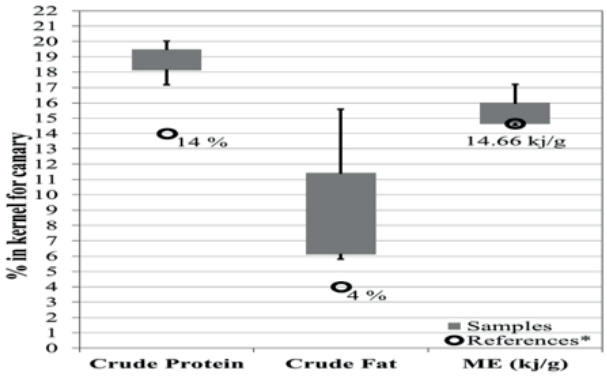

Figure 1. Comparison of nutrients of samples for canaries (n=35) with the requirements of an adult canary for maintenance. *Reference values were adapted from Orosz (2014) and Harper and Turner (2000).

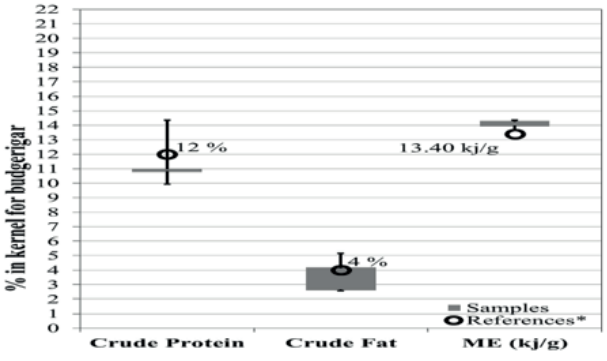

Figure 2. Comparison of nutrients of samples for budgerigars (n=35) with the requirements of an adult budgerigar for maintenance. *Reference values were adapted from Orosz (2014) and Harper and Turner (2000).

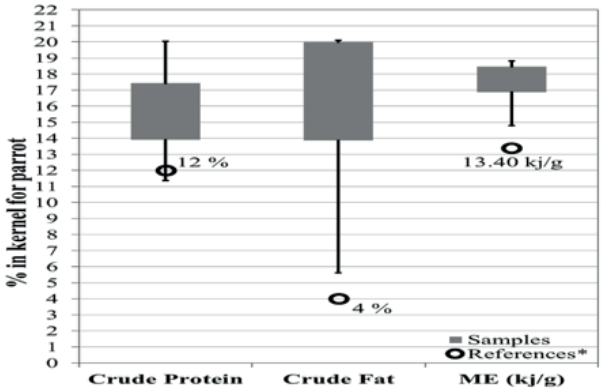

Figure 3. Comparison of nutrients of samples for parrots (n=35) with the requirements of an adult parrot for maintenance. *Reference values were adapted from Orosz (2014) and Harper and Turner (2000).

Extraction and detection of aflatoxins

For extraction of aflatoxins, 25 g of each sample, 125 ml of methanol-water (70:30 v/v) and 5 g NaCl were mixed and homogenized for 2 min/22000 rpm. This mixture was filtered with a filter paper and 15 ml of filtrate was mixed with 30 ml of pure mater in a glass beaker. This solution was filtered with Whatman microfiber filter paper (pore size 1.6 µm). 15 ml of filtrate was run through immunoaffinity column (EASIE-EXTRACT® AFLATOXIN) at 1-2 drop flow rate in a sec. Then, the column was washed with ultra-pure water (10 ml). HPLC-grade methanol was dropped (1-2 drop/sec) through to resolve linked aflatoxins and diluted by adding 10 ml ultra-pure water. Bounded aflatoxins to column were eluted with 1 ml HPLC-grade methanol and diluted with ultra-pure water (1 ml). 6:2:3 (v/v/v) rate of water/acetonitrile/methanol was passed (1ml/min) for the mobile phase. At 360 nm and 460 nm for excitation and emission respectively, levels of aflatoxins were determined on fluorescence detector (FLD) with COBra cell to derivate. The mobile phase was started up at a flow rate of 1 ml water/acetonitrile/methanol (6:2:3 v/v/v) in a min. and detection was observed with excitation at 360 nm and emission at 430 nm on the detector (FLD) duplicated with COBrA cell for derivatization.

Statistical analyses

Means of all data and standard deviation (SD) were calculated by using Excel (MS Office 2013).

Results

Nutritional contents

Samples for canary had the highest mean of CP content in all seed mixture samples as shown in Table 1. The contents of CP in all samples for canary were measured higher than the reference value as given Figure 1. Data mean of CF was 10.06% which is higher than the reference value for an adult

Table 1. Nutritional composition of samples with regard to the bird species

Samples	Crude protein (%)	Crude fat (%)	Soluble Carbohydrate (%)	Crude fibre (%)	Ash (%)	Moisture (%)	ME (kj/g)
Canary feed (n = 35)	18.84 ± 1.16	10.06 ± 4.12	49.03 ± 4.21	6.08 ± 0.46	4.98 ± 0.40	11.01 ± 0.75	15.65 ± 1.10
Budgerigar feed (n = 37)	11.41 ± 1.71	3.65± 1.10	62.52 ± 3.08	6.53 ± 0.43	2.93 ± 0.66	12.97 ± 1.19	14.10 ± 0.30
Parrot feed (n = 30)	15.53 ± 3.34	15.35± 6.01	50.07 ± 7.63	5.54 ± 0.51	2.87 ± 0.81	10.65 ± 1.02	17.29 ± 1.59

ME, metabolizable energy. Data (%) in fresh kernel.

canary. Standard deviation value was determined at the level of 4.12%, because of the wide range of CF contents in samples. Figure 1 had shown that all of samples for canary had contained higher CF than the reference value of 4%. Because of low carbohydrate content, data mean of ME levels in samples was slightly higher than the reference value of 14.66 kj/g.

Table 2. Results of microbiological contamination of pet bird feed

Samples (n=102)	Microbiological contamination (Mean ± SD; log cfu/g) Coliform	Salmonella in 25 g	Mould
Canary feed (n = 35)	3.25 ± 0.11 (n = 14)	<10 (n = 35)	4.10 ± 0.67 (n=35)
Budgerigar feed (n = 37)	3.19 ± 0.16 (n = 15)	<10 (n = 37)	4.61 ± 0.56 (n=37)
Parrot feed (n = 30)	3.43 ± 0.58 (n = 30)	<10 (n = 30)	4.46 ± 0.41 (n = 30)
Total (n = 102)	3.34 ± 0.43 (n = 59)	<10 (n = 102)	4.39 ± 0.56 (n = 102)

- : below the limit of detection

Data mean of CP contents in samples for budgerigar was approximately between 10 and 12%. But it was under the reference level of protein requirement for budgerigar. Only one of all samples had higher CP content than the reference value (Figure 2). As shown in Table 1 and Figure 2, both CF contents in all samples and the data mean were slightly lower than the reference value of 4% for budgerigars. Carbohydrate content mean was highest in budgerigar seed mixtures. The levels of ME in all samples were calculated between 13.66 and 14.36 kj/g, and the mean of all ME data was higher the reference value of 13.40 kj/g (Figure 2).

In samples for parrots, data mean of CP was measured higher than the reference value of 12%. In one of all samples, CP content was 11.37% and lower than the reference value. All samples for parrots had a wide range of CF content between 5.61 and 20.11%, as shown in Figure 3. So, CF contents of all samples and data mean were higher than the reference value of 4% for maintenance of parrots. Levels of ME in seed mix samples were calculated at a range of between 14.80 and 18.82 kj/g. When compared with the reference value of ME for parrots, all seed mixture samples had higher levels of ME (Figure 3).

Microbiological results are presented in Table 2. Out of the 102 feed samples, 58% were contaminated with Coliform with the level ranging from 2.5 to 4 log cfu/g. Mould was iso-

Table 3. Limit of detection and recovery results of aflatoxins during analysis

Feeds	Limits of detection (ppb)				Recoveries of method (ppb)			
	AFB1	AFB2	AFG1	AFG2	AFB1	AFB2	AFG1	AFG2
Budgerigar feed	0,4	0,4	0,5	0,5	94	91	93	78
Canary feed	0,2	0,4	0,3	0,5	89	90	81	82
Parrot feed	0,6	0,4	0,8	0,8	93	87	85	78

lated from 100% of the samples with ranging from 3 to 5.5 log cfu/g. All of the parrot feeds were contaminated with Coliform (100%), while budgerigar and canary feeds were less frequently contaminated with this bacteria (~40%, for each). The mean level of contamination of *Coliform* and mould were highest in parrot feed (3.43 log cfu/g and 4.46 log cfu/g, respectively). However no statistically significance was found between the data mean of *Coliform* and mould counts among 102 feed samples. None of the samples were contaminated with Salmonella spp.

Aflatoxin B1, B2, G1 and G2 results

Among the 102 feed samples no aflatoxin (B1, B2, G1 and G2) was detected. Limits of detection and recovery averages of method applied in samples were shown in Table 3.

Discussion

Nutritional analyses

The results had shown that the protein contents of unpacked seed mixtures for adult canaries and parrots were respectively 34% and 29% more than their requirements. However, in the results of budgerigars, protein contents was 5% lower. Koutsos et al. (2001b) reported that increasing crude protein level (from 11 to 70%) of feed caused the liver damage and influenced the enzymes of liver and kidney of adult parrots. Besides, it is important how much of protein in seed mixture was qualified and essential amino acids. The deficiency of some amino acides results in decreasing body weight and molting with stress in pet birds. To prevent the amino acids deficiency, supplemental protein sources (etc. nectar, pollen, fruits) were recommended for parrots (Koutsos et al 2001a, Brue 1994).

In this study, despite the low fat content of seed mixtures for budgerigar, it is assumed that the most of the energy requirement might be met with carbohydrate. But, in seed mixtures for canaries and parrots, crude fat contents were observed approximately 2.5 and 3.5 times more than their requirements, respectively. High fat content and energy density in seed mixtures may cause the metabolic diseases such as obesity and arteriosclerosis in pet bird, especially parrots (Bavelaar and Beynen 2004).

However, some of the studies showed the ability of bird to regulate their energy requirement by stopping feed intake (Koutsos et al 2001b). Previous studies suggested that feeds consist of seed mixtures had higher fat content and energy density when compared with packed and well-formulated feeds (Werquin et al 2005, Brightsmith 2012). The results of fat content in canary and parrot feeds have spread to a wide range (Figure 1 and Figure 3), which might reflect the unpacked seed mixtures have a non-standardized formulation.

Microbiological analyses

In this study Coliform bacteria were the most frequently isolated in parrot feeds. Level of Coliform contamination was within the equal range among the contaminated samples. The absent of Salmonella sp. among the samples in this study revealed that the risk to exposure to this pathogen through feed is low for pet bird. Despite the high frequency of mould contamination in the present study (100%; 3.34±0.43 log cfu/g), no aflatoxin was determined. The absent of aflatoxin is suitable with the current regulation of the Republic of Turkey Ministry of Food, Agriculture and Livestock (Official Journal 2014).

Most of the studies in Turkey focused on poultry feed collected from farm or feed producer, and found several mycotoxin including aflatoxin (Oruç et al 2012, Bilal et al 2014). Hence, the only available bird feed survey in Turkey reported low content (0.0-9.2 ppb) of aflatoxin (72%) (Oruç et al 2001). Moulds produced different levels of aflatoxin were detected in bird feeds worldwide. In canary and parrot feeds in Portugal, mould contamination (2 log cfu/g) was lower than this study, and no aflatoxin contamination was reported (Martins et al 2003).

In the study of Scudamore et al., 370 ppb AFB1 was detected in one of the bird food (groundnut) out of 100 pet food samples (1%) (Scudamore et al 1997). Occurrence of total aflatoxin in bird feed was 94.7% with the range of between 3.09 and 167.5 ppb in Brazil (Scussel et al 2006). The survey in Belgium reported one of 10 commercial parrot feeds (10%) contaminated with AFB1 (Li et al 2013).

High level of contamination can cause death of animals and pet birds, as small animals should be considered as more susceptible to those mycotoxins than bigger ones (Lightfoot and Yeager 2008). However, individual sensitivity of birds might also have an effect on the results regarding to acute or chronic exposure to mycotoxins. Nevertheless, microbiological results in this study are found in the satisfactory limits.

Conclusion

In conclusion, though it seems to satisfy the base nutrient requirements nutritional imbalance in formulation of unpacked seed mixtures may induce the metabolic diseases which decrease the lifespans of pet birds. However, the absent of Salmonella sp. and aflatoxins is promising for the health of pet bird.

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