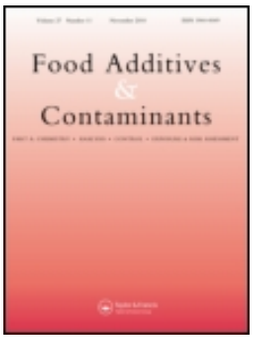

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Occurrence of aflatoxins in layer feed and corn samples in Konya province, Turkey

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The natural occurrence of aflatoxin was investigated in layer feed and corn samples brought to Konya Veterinary Control and Research Institute Laboratory between 15 April and 15 December 2002. Seventy-eight samples (52 feeds, 26 corn samples) were analysed for total aflatoxin ($B_1+B_2+G_1+G_2$) by an ELISA screening method. Aflatoxin contamination was determined in 37 feed samples (71.1%) and 15 corn samples (57.7%), with a range of 1.5–133 $\mu g\,kg^{-1}$. However, a majority of the aflatoxin contamination was less than 5 $\mu g\,kg^{-1}$ (50% within the positive samples). Two feed samples and two corn samples exceeded the maximum tolerated levels in feed (20 $\mu g\,kg^{-1}$) and feedstuffs (50 $\mu g\,kg^{-1}$) for total flatoxin.

Keywords: aflatoxin, ELISA, layer feed, corn, Konya province

Introduction

Aflatoxins ($B_1+B_2+G_1+G_2$) are a group of heterocyclic metabolites produced by storage fungi of the genus *Aspergillus*, particularly *A. flavus* and *A. parasiticus*. Aflatoxins (AF) have been a major concern as human hepatocarcinogens and as substances with potential deleterious effects on livestock health and productivity. Aflatoxicosis in poultry causes listlessness, anorexia with lowered growth rate, poor feed utilization, decreased egg weight and production, decreased body weight gain, increased susceptibility to environmental and microbial stresses, and increased mortality (Kubena *et al.* 1993, Oguz and Kurtoglu 2000, Salwa *et al.* 2000). The most prominent manifestations of aflatoxicosis in layers are reduced egg production and egg weight, increased liver fat, and the alterations in serum biochemical and haematological parameters (Leeson *et al.* 1995).

AF contamination in layer feed carries over to eggs, particularly aflatoxin B_1 and aflatoxicol. The occurrence of AF residues in eggs becomes particularly important as a potential human health hazard (Oliviera *et al.* 2000) since AF are implicated in the aetiology of hepatic cancer (Wild *et al.* 2000, Wang *et al.* 2001). AFs or their metabolites are detected in all egg components as early as 10 h after ovulation and 14 h after oviposition. The concentration of label declined in albumen after 48 h while levels in the yolk and shell membranes increased (Sawhney *et al.* 1973). The carry-over of AF from layer feed to eggs has been investigated and the transmission ratio (concentration of AF in feed over residual AF or their metabolites) ranged from 66 000:1 to 2200:1 (Oliviera *et al.* 2000).

The carry-over of AFs from the layer feed to the egg also results in significant economic losses because of their embryotoxic–teratogenic effects. AF residues in eggs have been reported to cause a variety of disturbances, from early embryonic death to organ malformation, depending on the residual levels of AFs and their metabolites in eggs (Kemper and Luepke 1986). Trace amounts of AF found in fertilized eggs might cause functional defects in the immune system of the developing embryo and such chickens might show serious immunodeficiency (Dietert *et al.* 1985). These effects might lead to serious economic losses in poultry production by decreasing hatchability

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(Celik *et al.* 2000). Embryonic deaths have been reported when eggs contained up to 5 ng AFB_1 and embryonal mortality reached 50% within the fertilized eggs at levels of 10–20 AFB_1/egg; mortality values reached 100% at levels of 100 ng AFB_1/egg (Sur and Celik 2003).

The continuous control of layer feed for AF contamination is particularly important since the negative effects of AF in the layer feed for human and animal health and their embryotoxic effects. Many studies have been performed to determine AF contamination levels in poultry feeds in different countries and to minimize their toxic effects on human and animal health. Surveys in Turkey (Basaran *et al.* 1986, Acet *et al.* 1989, Ozkazanc *et al.* 1992, Nizamlioglu 1996) demonstrated that the AF concentrations in feeds and feedstuffs ranged from 1 to 80 $\mu g\,kg^{-1}$ and these levels were generally less than 30 $\mu g\,kg^{-1}$. The WHO (1998) report and other studies (Kichou and Walser 1993, Pitet 1998) listed the AF contaminations in feed and feedstuffs in 30 different countries and reported 1–700 $\mu g\,kg^{-1}$. Generally, these occurred at less than 50 $\mu g\,kg^{-1}$. AF contamination has been reported at higher levels (1–6000 $\mu g\,kg^{-1}$) in warm and humid tropical climates such as India (Jindal *et al.* 1993), Indonesia (Purwoko *et al.* 1991, Bahri 1998), Egypt (Hegazy *et al.* 1991) and Nigeria (Shetty *et al.* 1987). Producers and scientists aim to develop effective prevention and decontamination technologies (Oguz and Kurtoglu 2000) and governments also want to limit and control AF levels in feed and feedstuffs by legislation.

Corn is grown in a mild climate and relatively high humidity. It is also a cereal commonly used in poultry feeds (50–60% in the ration). Survey studies showed that the levels of AF contamination in corn ranged from 0.1 to 80 $\mu g\,kg^{-1}$ (Jelinek *et al.* 1989), from 8 to 369 $\mu g\,kg^{-1}$ (Bahri 1998), from 0.1 to 16.4 $\mu g\,kg^{-1}$ (Scudamore and Patel 2000), from 38 to 460 $\mu g\,kg^{-1}$ (Ono *et al.* 2001), and from 0.4 to 128 $\mu g\,kg^{-1}$ (Wang *et al.* 2001). The nutritional and economic importance of corn, together with the risk of a serious health hazard due to AF association, emphasizes the need for rapid analyses and continuous control in the field, as provided by rapid and accurate immunological assays (Ono *et al.* 2001). In the Konya province of Turkey, layers (approximately 5 million hens) are commonly grown. Therefore, in the present study, the frequency and range of AF contamination in layer feed and corn samples is reported.

Materials and methods

Sampling

The samples analysed were provided by producers (poultry farms and feed industries). Fifty-two layer feeds and 26 corn samples were brought by producers to the Konya Veterinary Control and Research Institute Laboratory between 15 April and 15 December 2002 for toxicological analyses. These samples were analysed individually (without subsampling) for total AF ($B_1 + B_2 + G_1 + G_2$).

Recovery and detection limit

Recovery for the extraction method was determined by adding a known amount of total AF (10, 20, 30, 40 $\mu g\,kg^{-1}$) to the feed and corn samples. The samples were analysed. Mean recoveries were 79% for feed and 83% for corn. The detection limit of the ELISA reader was determined by the addition of different AF levels (0.5, 1, 1.5, 2 and 2.5 $\mu g\,kg^{-1}$) to corn samples. After analysis it was 1.5 $\mu g\,kg^{-1}$.

Aflatoxin analysis

The samples were analysed by an ELISA (RIDASCREEN® FAST Aflatoxin) test kits (r-biopharm AG, Darmstadt, Germany). The detection limit, recovery and accuracy of the AF method in feed were confirmed by a TLC-Scanner (Camag III, Basel, Switzerland) in prior analyses. When compared with the TLC-Scanner, this ELISA method was thought of as more practical and less time-consuming for routine AF analyses in the laboratory.

Ground samples (5 g) and 25 ml methanol–water (70:30 v/v) were vigorously shaken for 3 min and filtered through Whatman No. 1 filter paper. Then, 1 ml of the filtrate was diluted with 1 ml distilled water and 50 µl were added per well. Then 50 µl standard and prepared sample were added to separate wells and 50 µl enzyme conjugate and 50 µl AF antibody were added to each well, respectively, and mixed and incubated for 10 min at room temperature (20–25°C). Next, the wells were dumped and washed three times with distilled water. Then, 100 µl substrate/cromogen were added to each well and mixed and incubated

for 5 min at room temperature in the dark, followed by the addition of 100 µl stop solution to each well and mixing. The AF concentration was determined by plotting absorbance at 450 nm and was read within 10 min (Bio-Tek Instruments EL 311 SX, Switzerland). The AF concentrations ($\mu g\,kg^{-1}$) to the extinction of each sample were extrapolated from the calibration curve. Alternatively, the software program can be used for data reduction.

Results and discussion

The AF concentrations in the test samples are summarized in table 1. The AF contamination was determined in 37 layer feed samples (71.1%) and 15 corn samples (57.7%), range 1.5–133 $\mu g\,kg^{-1}$. Generally, the AF contaminations were less than 5 $\mu g\,kg^{-1}$ (50% within the positive samples). Two samples from layer feed exceeded the maximum tolerable AF levels in feed (i.e. 25.8 and 46.8 $\mu g\,kg^{-1}$). Similarly, two corn samples also exceeded the legal AF levels in feedstuffs (i.e. 67.4 and 133 $\mu g\,kg^{-1}$).

AF contamination in feed and feedstuffs (especially corn) has become a major concern to the developed poultry industry as well as those of developing countries because of the apparent potential for widespread occurrence yet its clinically unapparent effect through continued low levels of intake (Hegazy *et al.* 1991). Generally, AF is common in warm and humid climatic conditions such as those in Latin American, Asia and Africa, and in certain parts of Australia; the reported levels in feed and feedstuffs are higher (1–6000 $\mu g\,kg^{-1}$) in these countries (Shetty *et al.* 1987, Jindal *et al.* 1993, Leeson *et al.* 1995, Bahri 1998, WHO 1998, Wild *et al.* 2000, Wang *et al.* 2001, Abdulrazzaq *et al.* 2002). AF can occur along the coast of Turkey depending on the climatic season and feed/feedstuff production technologies. The reported levels of AF contamination in feed resulting in significant adverse effects varied from 60 to 800 $\mu g\,kg^{-1}$ depending on the period of exposure in poultry. The clinical, haematological–biochemical and histopathological changes have been reported to be up to 100 $\mu g\,kg^{-1}$ AF in feed in poultry (Abdelhamid *et al.* 1994, Oguz *et al.* 2000, Raju and Devegowda 2000, Oguz *et al.* 2002). However, immunological parameters can be affected at the lower doses of AF (below 100 $\mu g\,kg^{-1}$) in feed (Oguz *et al.* 2003). For this reason, governments, scientists and producers aim to develop effective prevention and decontamination strategies and technologies.

Table 1. Aflatoxin ($B_1+B_2+G_1+G_2$) contamination in layer feed and corn samples in Konya province, Turkey.

Aflatoxin ($\mu g\,kg^{-1}$)	Feed samples (%)	Corn samples (%)
< 1.5 (non-detectable)	15 (28.9)	11 (42.3)
1.5–5	23 (44.2)	3 (11.5)
5–10	7 (13.5)	4 (15.4)
10–15	3 (5.8)	– (0)
15–20	2 (3.8)	1 (3.9)
> 20	2 (3.8)	7 (26.9)
Total	52 (100)	26 (100)

In layers, the AF contamination in feed is important, not only to poultry, but also to public health due to AF residues in eggs. Many countries have regulated AF concentrations in feed and feedstuffs and these limits have been strictly controlled. In Turkey, the maximum tolerable levels in terms of total AF are 20 $\mu g\,kg^{-1}$ for layer feed and 50 $\mu g\,kg^{-1}$ for feedstuffs (Ministry of Agriculture 1991). The levels of contamination in feed and corn samples from this study agree with previous surveys in Turkey indicating 1–80 $\mu g\,kg^{-1}$ (Basaran *et al.* 1986, Acet *et al.* 1989, Ozkazanc *et al.* 1992, Nizamlioglu 1996). It is important to find AF levels lower than the permitted levels in feed and feedstuffs. In the present study, four samples exceeded the legal upper AF levels (table 1). The percentage of positive samples was high (i.e. 66.7% within the total samples). The reason for this high frequency of contamination might be related to the limited numbers of samples screened from poultry farm producers. However, note that most of the contamination levels were lower than 5 $\mu g\,kg^{-1}$ in feed (73.1%) and in corn (53.8%) within the total samples analysed. Although this study is not an exhaustive survey of feeds, the samples analysed come from all the poultry farms in Konya, and thus reflect the AF contamination in Konya province.

Regarding AF transmission ratios (Qureshi *et al.* 1998, Oliviera *et al.* 2000), parent hens should be fed rations containing AF at levels less than 150 $\mu g\,kg^{-1}$ for safe eggs in terms of embryotoxicity; abnormality and mortality can occur in chicken embryos when AF residues are available at levels greater than 5 ng/egg or 0.1 $\mu g\,kg^{-1}$/egg (Sur and Celik 2003). Since AF are potent hepatocarcinogens for human and animals and cause significant eco-

nomic losses, greater attention should be paid to the occurrence of AF in feeds and feedstuffs.

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