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Aflatoxin B1 contamination in local and industrial eggs measured by ELISA technique in Mazandaran

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ABSTRACT

Aflatoxins are a highly toxic and carcinogenic mycotoxins which produced by Aspergillus *species. This study was done to determine the levels of aflatoxin contamination in feed, industrial and local eggs in west region of Mazandaran in Iran. Sampling was done from all local and industrial units of egg production randomly and simultaneously. 1400 industrial eggs from seven different zones with 5 replicate (in each replicate 40 eggs) and 700 traditional or local eggs from 7 points in 5 replications were collected. In addition 4 poultry feed samples with 5 replicate (Corn, Barley, Pletted diet and soybean meal) were collected. Aflatoxin B1 in samples was measured by ELISA method.* Aflatoxin B1 ELISA *Test Kit of Europroxima that used in this research is based on competitive enzyme immunoassay for the quantitative analysis of Aflatoxin B1 in cereals and feed. Tests and standards were performed duplicated. Results showed aflatoxin contamination levels in local eggs were more than industrial samples numerically (P > 0.05). Highest aflatoxin contamination was seen in Chalous local eggs (0.107 ng /ml) and lowest contamination was seen in industrial egg from Sattari farm (0.050 ng /ml) respectively. These values were lower than allowed limit (12 ng /ml). There was no significant difference between industrial eggs from different farms of Mazandaran (P>0.05).*

Keywords: aflatoxin, mycotoxins, local egg, industrial egg, ELISA.

INTRODUCTION

Aflatoxins are a distinct group related to mycotoxins. They are poisonous and produced by a group of fungi called as *Aspergillus flavus* and *Aspergillus parasiticus* species. Only some strains of these two species are able to produce toxin and therefore belonging to the *flavus* and *parasiticus* species cannot be a good reason for toxin production. The fungus under favorable conditions of moisture and temperature will be able to develop on special foods along with producing toxin. Thus, Aflatoxins are secondary metabolites of fungi.

In yeast and some other organisms, primary metabolites are compounds that are essential for growth and reproduction. Secondary metabolites are produced at the end of the logarithmic phase of growth and are important in the growth and metabolism of the organism. But their actual role in growth is not clear [1]. More than 18 types of aflatoxin are known so far which four of them are produced naturally in the name of aflatoxin B1, B2, G1 and G2. Aflatoxin B1 is the most dangerous among them [2, 3, 4].

Aflatoxins are a municipal health concern and can damage food ingredients in all stages of production, processing, transportation and storage of food [1]. These toxins are found in a variety of food and animal feed. Aflatoxin contamination of foods typically caused by improper maintenance or during post-harvest in food production. Foods could be contaminated by *Aspergillus* in different ways. Consumption of foods that contaminated by aflatoxin M1 may cause the presence of this toxin in milk of livestock and poultry meat and eggs [5, 6].

Fungi prefer to grow on foods that are rich in carbohydrate. It is observed that the certain mycotoxins could be found in particular foods [7]. Aflatoxin contamination has been reported frequently for oil seeds like groundnut. There are numerous reports of aflatoxins in cereals as well; however there has been usually less report for soybean meal. Studies have shown a direct relationship between food composition and mycotoxin production in animal foods. If one person consumes less than 10ng of aflatoxin B1 per day per kg of body weight for a long period of time, he/she could experience terrible temporary effects, but if consume 50 micrograms, important clinical and epidemiological effects will be happened. Since aflatoxin M1 is less toxic it must be in high levels present in foods to act as an important toxin [8]. Routine methods used for measuring aflatoxin in foods are qualitative methods (such as immunoassays) or quantitative methods (such as HPLC). The aim of this study was to investigate aflatoxin contamination in local and industrial eggs in west of Mazandaran (Nowshahr and Chalous) using ELISA method**.**

MATERIALS AND METHODS

Sampling

In this study 1400 industrial eggs from seven different zones with 5 replicate (in each replicate 40 eggs) and 700 traditional or local eggs from 7 points in 5 replications were collected. In addition 4 poultry feed samples with 5 replicate (Corn, Barley, Pletted diet and soybean meal) were collected. Aflatoxin concentration in these samples was determined via ELISA techniques.

Laboratory analysis

In this study, we used Europroxima kit and take the following protocol for measuring aflatoxin B1 in the experimental samples:

Before testing the equipment and materials needed for experiments washed with detergent and distilled water, then sterilized with autoclave. Afterward the collection was done randomly from different sites it happened simultaneously we in 6 days. Aflatoxin B1 in samples was measured by ELISA method. In these test antibodies against aflatoxin B1 on the bottom of the wells is attached to the kit. Tests and standard double (Duplicate) was performed. Consequently well was sufficient for 40 tests. Kit used in this study specifically was Europroxima kit to measure aflatoxin B1 in food.

Statistical Analysis

Data were subjected to analysis of variance in a completely randomized design using the SAS program General Linear Model (GLM) procedure (SAS, 9.1, 2005). Significant means were compared using the least square means method. Mean differences were considered significant at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS

One thousand and four hundred industrial eggs from seven different points with 5 replicate, including Yadu'llah Nowshar farm (1), university shop for first purchasing (2), university shop second purchase (3), Sattari farm (4), Niloo company of Akbarpuor (5), Naymaey Nowshahr farm (6) and Davoud farm (7) was carried out and furthermore 600 local eggs from 7 region including Chalous (two region 1 and 2), Nowshahr (two region 3 and 4), Kelardasht (5), Kojour (6 and 7) were tested using ELISA technique. The results are presented in table 1. Highest aflatoxin contamination was seen in Chalous local eggs (0.107 ng /ml) and lowest contamination was seen in industrial egg from Sattari farm (0.050 ng /ml) respectively. These values were lower than allowed limit (12 ng /ml). There was no significant difference between industrial eggs from different farms of Mazandaran ($P>0.05$). There also was no significant difference between local eggs from different regions of Mazandaran ($P>0.05$). Numerically the local eggs had higher values of aflatoxin contamination in compare to industrial eggs. Whereas in local eggs the aflatoxin concentration was not less than 0.080 (ng/ml) but in industrial eggs the highest value was 0.081 (ng/ml).

Table 1. Aflatoxin concentration in eggs collected from industrial and local farms in west region of Mazandaran (Iran)

Aflatoxin (ng/ml)	*Treatments*								Sig.
	1	2	3	4	5	6	7	SEM	P-Value
Industrial egg	0.0726	0.0744	0.0578	0.0502	0.0816	0.0654	0.0642	0.0102	0.389
Local egg	0.1070	0.1000	0.0800	0.0828	0.0842	0.1042	0.0902	0.0106	0.401

The results of aflatoxin contamination in 5 common feed samples have been shown in table 2. Least square difference of means showed no significant difference between corn, barley, soybean meal or pelleted diet in afalatoxin concentration. Although afaltoxin levels in feeds was higher than local and industrial eggs, whereas pelleted dite had the highest contamination (0.411 ng/ml) and soybean meal had the lowest value. Contamination level of aflatoxins in pelleted diet samples was not significantly more than corn, barley and soybean meal (P >0.05).

Table 2. Aflatoxin concentration in 5 common feed of poultry ration in Iran

	Feed ingredient					
	Barley	Corn	Pellet	Soybean meal	SEM	P-Value
Aflatoxin (ng/ml)	0.3192	0.3280	0.4114	0.2696	0.070	0.570

DISCUSSION

According to the current results observed that most of aflatoxin contamination was 0.083 ng /ml and lowest contamination was 0.050 ng /ml respectively. These values are lower than the usual limit of aflatoxin in feed (12 ng/ml). Mycotoxins are worldwide problem and according to the Food and Agriculture Organization (FAO) information. Approximately 25 percent of the world's grain crops are contaminated with mycotoxins. Particularly aflatoxins and mycotoxins, according to the WHO report, are contributing factors in food borne disease which is so important. In one study on the slaughterhouses in Ahwaz found that 37.5 percent of livers, 22.5 percent of breast and thigh muscles of slaughtered chickens were contaminated with aflatoxin. Pectoral muscle and liver had the highest and lowest prevalence of aflatoxin B1 and M1, respectively [9]. Statistical analysis showed that the average amount of aflatoxin B1 and M1 residues in the liver was significantly higher in breast and thigh muscles of chickens which were slaughtered [9]. The toxins produced by fungi can cause disease in animals and birds and their direct or acute symptoms can be observed. They also can act by reducing the immune system of livestock and poultry and susceptible them to the pathogen, or could be entered in the human food chain through milk, meat and eggs, and cause danger to human health [10]. In this experiment it was shown that aflatoxin contamination of the industrial eggs was much more than local and traditional eggs. Effects of aflatoxins on animals will vary depend on the concentration and duration of contact, breed, and diet. Natural zeolites are good additives which could be use for controlling fungal toxins. The surface of this material can be more active and produce more electrical charge, which increases its efficiency in absorbing mycotoxins [11]. Aflatoxin control is necessary because they can transfer to animal products. Aflatoxin M1 is derived by Aflatoxin B1 [12]. Aflatoxin contamination of corn is a major problem in humid regions [13]. Currently preventing and neutralizing the toxins in animal feed and human food industry is an important issue facing the world and necessarily to protect public health they should be removing from food [14, 6, 15].

CONCLUSION

Due to favorable conditions for fungal growth and mold outbreak in northern Iran, along with the high humidity and high temperature, the presence of fungi and toxins (aflatoxins) obtained from food sources, especially soybean meal for poultry not unexpected. With proper planning, management, maintenance, storage, handling and preparation of food can be achieve to decreasing aflatoxin in poultry.

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