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A COMPARATIVE INVESTIGATION ON EGG YOLK TOTAL ANTIOXIDANT CAPASITY RELATIVITIES TO MYCOTOXINS AFLATOXINS

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Abstract

Mycotoxins, along with other fungal metabolites such as antibiotics, alkaloids, etc., are compounds that are produced by fungal cells in the final stages of filamentous fungal growth. Among mycotoxins, aflatoxins are known as the most well-known mycotoxins due to reasons such as their relatively long history of widespread use in experimental and abundant research in nature). Samples were performed by the test kit and the ELISA reader according to the kit instructions. Finally, the collected data were analyzed using SPSS software and descriptive statistics (contamination ratio, mean and standard deviation) and one-way analysis of variance. In the amount of aflatoxin in all egg yolk samples of the studied brands, the minimum value was 0.000ppb and the maximum was 229.44 ppb in the p / p analysis and also the minimum value was 0.000ppb and the maximum was 259.78 in the Cub analysis. The levels of mycotoxins in the present study were in the range of natural presence of this toxin in the maximum acceptable amount of 500 ppb infected in the country. Therefore, the transfer of mycotoxin metabolites to eggs has been possible, and therefore control of mycotoxin contamination in laying hen diets is recommended to avoid the presence of mycotoxins in eggs intended for human consumption.

Keywords: Mycotoxin, Aflatoxin, egg yolk, Antioxidant capacity.

INTRODUCTION

Mycotoxins are important environmental pollutants that can be produced on a variety of grains, nuts and other plant components. Human and animal contact with these toxins is through oral, inhalation, or direct contact, and even very small amounts of these compounds can be hazardous. Toxin-producing fungi can grow and produce toxins on a wide range of substrates such as growing leaves and stems, seeds, fruits, and plant and animal foods (4-2). However, some substrates are more suitable for producing the maximum amount of toxin, which may be due to the type of chemical composition. In this regard, the specificity of the substrate for the fungus is one of the most important aspects of research on mycotoxins in the field of animal and human food contamination. Mycotoxin-producing fungi are able to contaminate agricultural products at different stages of planting, holding, harvesting and storage (4).

Today, most mycotoxins can be identified at an acceptable level in terms of legal, veterinary, and medical needs. Urine and milk are important. And most studies have focused on

increasing sensitivity, accuracy and reproducibility and, most importantly, reducing analysis time (5).

Material and Methods

Materials

Materials in the test kit

Each kit made by the Dutch company Europroxima contains the following materials:

A 96-well microtiter (in 12 rows of 8) coated with antibody against AFB1. Seven vials containing AFB1 solution with concentrations equal to:

0ng / ml (standard zero), (0313 (ng / ml), 0/0625 (ng / ml), 0/125 (ng / ml), (0/25 (ng / ml, 0.5 / ng / ml), 1 (ng / ml) of AFB1.

One vial of conjugated solution is lyophilized

An antibody vial in the form of lyophilisation

One vial of 12 ml substrate solution

One 15 ml stopping solution vial containing normal sulfuric acid.

A 20 ml solution solution vial to dilute the sample and standard solution.

One solution vial with carpet volume of 30 ml.

Preparation of reagents

Before starting the test, some of the ingredients need to be prepared and some are ready to use. After washing, it has been concentrated 20 times and in order to use it, 2 ml of it must be diluted with 38 ml of distilled water and a new solution must be prepared for each use.

The buffer dilution is quadrupled and in order to use, 20 ml of it must be diluted with 60 ml of distilled water at room temperature and shaken vigorously. Conjugate solution and antibody solution are available in lyophilized kit and in order to use, 4 ml of dilution buffer should be added to them and shaken vigorously and stored in a dark place until use.The chromogenic substrate solution in the kit is ready to use and must be stored at room temperature before use.

Collection of samples

800 eggs from 34 brands were randomly selected and purchased each time with specific expiration dates. And the samples were collected during 3 seasons of the year and a total of 2400 samples were analyzed.

Sensitivity and specificity of the experiment

The sensitivity of the test varies between 0.5 and 1 ppb depending on the test matrix. The specificity of this test is based on interactions with aflatoxin B1, 100%, aflatoxin B2, 20%, aflatoxin G1, 17% and aflatoxin G2, 4%.6-3 TAC statistical analysis method (Dr. Shahriari Ahvaz to be replaced)

Measurement of total Antioxidant Capacity

This test is based on the regenerative capability of the sample to regenerate ferric ions to ferrous ions and to form a blue Fe2+-TPTZ complex, which can be evaluated by spectrophotometric method. In order to calculate the total antioxidant activity, first the standard diagram of absorption of different concentrations of iron was obtained and then using the line equation $Y = ax + b$, the total antioxidant capacity of unknown samples was obtained.

Statistical analysis method

The collected data were analyzed using SPSS software using descriptive statistics (contamination ratio, mean and standard deviation) and one-way analysis of variance.

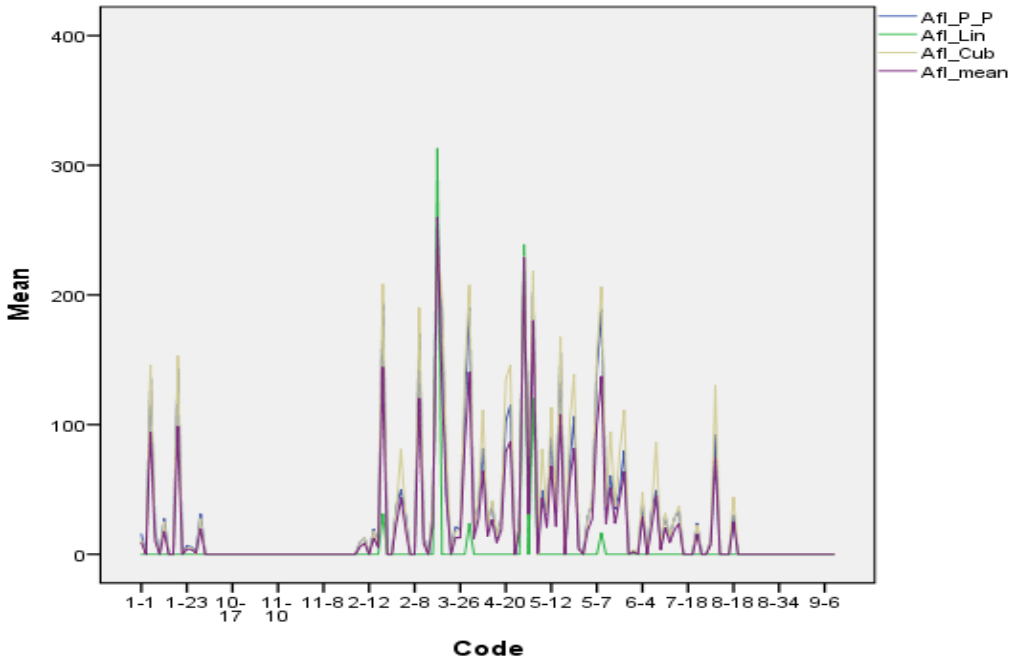
Results and Discussion

Evaluation of aflatoxin levels in all egg yolk samples

In the amount of aflatoxin in all egg yolk samples of the studied brands, the minimum value was 0.000ppb and the maximum was 229.44 ppb in the p / p analysis, as well as the minimum value of 0.000ppb and the maximum was 259.78 in the Cub analysis. The improved mean with 5% error (Trimmed mean 5%) at

20.2965 (P / P), 24.1445 (Cub) and 14.8137 showed the average measured aflatoxin values. The highest values were 237.09-207.51 and the average was 140.47-259.78 in samples 46, 24, 29, 44 and 36. Which include brands 4, 4, 2, 2 and 3. The lowest amount of aflatoxin measured was 0.00 in samples 153, 152, 151, 150 and 154, which includes brand 12.

Figure 1-1: Comparative diagrams of the average aflatoxin levels in yolk samples provided by the studied brands based on point of point, linear and cubic function.



Evaluation of the correlation between Pearson analysis and aflatoxin levels

The correlation between Pearson analysis and the measurement of aflatoxin levels by P / P and cub methods showed a statistical correlation (PC: 0.991). This correlation between the mean value of total aflatoxin measured by P / P (PC: 0.979) and Cub (PC: 0.969) was also statistically significant (Sig: 0.000). Therefore, it can be found that regardless of the analysis and processing of values measured by competitive indirect ELISA method, all data obtained by different methods of numerical functions in determining the amount of toxin will have the same behavior in numerical analysis with statistical methods. This view is also considered in the analysis of WIL COXON analysis between the levels of aflatoxin P / P and aflatoxin cub (Z: -6.887 / Sig: 0.000) as well as aflatoxin P / P and aflatoxin total mean (Z: -6.727 / Sig: 0.000) Also aflatoxin cub and mean aflatoxin total (Z: -7.048 / Sig: 0.000) have been observed and strongly confirms the above conclusion.

Evaluation of aflatoxin levels in all egg yolk samples

In the study of the amount of aflatoxin present in all egg yolk samples of the studied brands, the minimum value was 23.17ppb and the maximum was 252.80 ppb in the p / p analysis, and the minimum value was 35.50ppb and the maximum was 227.10 in the cub analysis. The average was 29.34 and the maximum was 239.95.

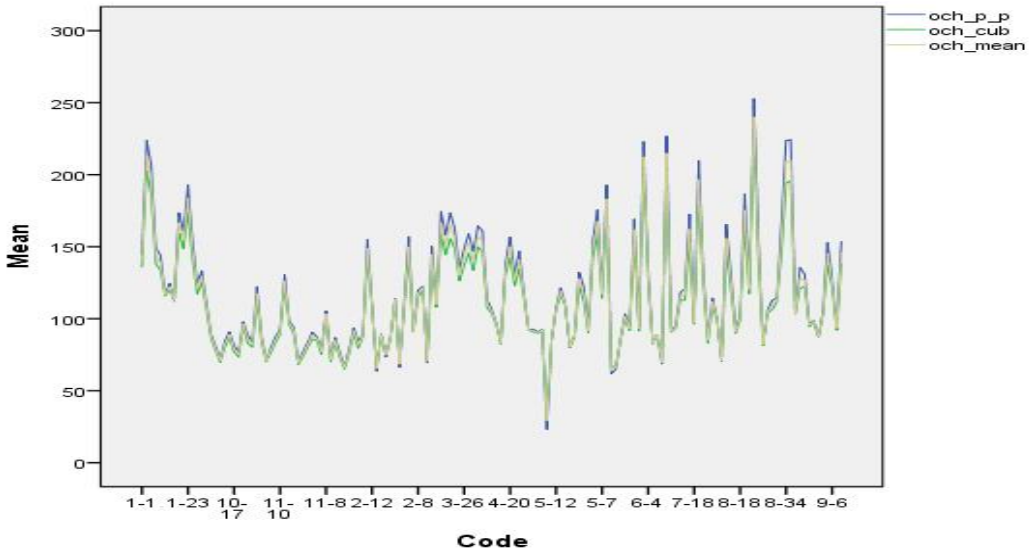
The improved mean with 5% error (Trimmed mean 5%) of 114. 294 (P / P), 108.5811 (cub) and 111.6994 showed the mean measured values of ochratoxin.

The highest amount of ochratoxin measured was 227.10 cub-195.88 and the average was 210.09-239.95 in samples 85, 11, 70, 111 and 104. Which include brands No. 7, 1, 6, 8 and 8.

The lowest values of ochratoxin measured were 90-67-50-35 and the average was 29.34-66.45

in samples 22, 74, 154, 58 and 75, which include brands 2, 6, 12, 5 and 6

Figure 1-2: Comparative graphs of the average aflatoxin levels in yolk samples provided by the studied brands based on point, point, linear and cubic function



Investigation of the correlation between the amounts of mycotoxins measured

Findings of the correlation between the amounts of mycotoxins measured with the amounts of fatty acids C4-C28(C14-C24) measured using wil coxon statistical analysis show that the mean amount of total aflatoxin is positively correlated with the following fatty acids and the number of their chain carbides, respectively (Table 1-1)

Table 1-1. Statistical findings of the Wilcoxon test in measuring the correlation between the average amount of aflatoxin and the concentration of fatty acids in egg yolk.

Test Statistics[a]

	PI-C14 - Afl_mean	PL-C15 - Afl_mean	PL-C16 - Afl_mean	PL-C17 - Afl_mean	PL-C18 - Afl_mean	PL-C20 - Afl_mean	PL-C22 - Afl_mean	PL-C24 - Afl_mean
Z	-6.270[b]	-6.549[b]	-1.545[b]	-6.206[b]	-1.656[b]	-6.573[b]	-5.656[b]	-6.624[b]
Asymp. Sig. (2-tailed)	.000	.000	.122	.000	.098	.000	.000	.000

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

C24>C20>C15>C14>C17>C22>C18>C16

Table 1-2. Statistical findings of Wilcoxon test in measuring the correlation of P/P measurement of aflatoxin and egg yolk fatty acids concentration.

Test Statistics[a]

	PI-C14 - Afl_P_P	PL-C15 - Afl_P_P	PL-C16 - Afl_P_P	PL-C17 - Afl_P_P	PL-C18 - Afl_P_P	PL-C20 - Afl_P_P	PL-C22 - Afl_P_P	PL-C24 - Afl_P_P
Z	-6.399[b]	-6.886[b]	-2.933[b]	-6.388[b]	-2.667[b]	-6.593[b]	-5.965[b]	-6.641[b]
Asymp. Sig. (2-tailed)	.000	.000	.003	.000	.008	.000	.000	.000

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

C24>C20>C15>C14>C17>C22>C18>C16

Despite this, this finding does not differ from the mean aflatoxin average compared to the fatty acids measured and correlates with the same values C14 -C24 (Table 1-2)

Species of the genus Aspergillus are the most common contaminants of various substrates and are widespread worldwide, especially in tropical and subtropical regions. Among the toxin species, Aspergillus flavus or Aspergillus parasiticus are of special importance due to their ability to produce aflatoxin, and about half of the strains of Aspergillus flavus and Aspergillus parasiticus are common in the environment. Various toxic metabolites are

produced by Flavus and Parasiticus species. Aflatoxins are a group of toxins containing approximately 20 toxic compounds, of which aflatoxin B1 is the most important (8). Aflatoxin B1 in the diet is metabolized in the liver after consumption and is secreted as aflatoxin M1 in the body's natural fluids and, due to its carcinogenic properties, mutagenicity and endangers public health (6). Due to the importance of fungal toxins in the human food chain in many countries to protect consumers from the harmful effects of mycotoxins in food, special regulations have been enacted.The General Directorate of Food and Drug Control in Iran has set the permissible limit of afatoxin B1 for animal consumption at 5 micrograms per kilogram and the total permissible limit for aflatoxins B1, B2, G1 and G2 at 20 micrograms per kilogram. The European Union and the Food and Drug Administration have also set the permissible levels of aflatoxins in various food products, including animal feed, in tables (4). In a study sent to colleagues (86-87) in Shiraz and its suburbs, a total of 428 samples including milk and animal feed were sampled in different seasons and tested by ELISA and TLC methods. The results showed that in 36.43% of the animal feed samples the level of aflatoxin B1 contamination was higher than the set limit. According to the study of Rahimi et al. (9-10) in Chaharmahal Bakhtiari province, out of 108 animal feed samples, 73 samples (67.6%) were in the range of aflatoxin B1 contamination in the range of 0.80 to 155.8 μg / kg feed. The mean contamination in winter and summer were 32.65 and 16.76 μg / kg, respectively (3).

Egg yield and egg weight are not significantly affected by dietary treatments of 50 and 100 aflatoxins per feed, so the limited range of aflatoxins measured in the present study from 140 to 237 ppm could indicate the significant risk to the material health cycle. Food threatens humans when consuming eggs. Regardless of the choice of analysis and processing of values measured by competitive indirect ELISA method, all data obtained by different methods of numerical functions in determining the amount of toxin will have the same behavior in numerical analysis with statistical methods. This view is also considered in the analysis of WIL COXON analysis between the values of aflatoxin P / P and aflatoxin cub (Z: -6.887 / Sig: 0.000) as well as aflatoxin P / P and aflatoxin total mean (Z: -6.727 / Sig: 0.000) Also aflatoxin cub and mean aflatoxin total (Z: -7.048 / Sig: 0.000) have been observed and strongly confirms the above conclusion.

Diets contaminated with aflatoxins and aflatoxins, such as commercial poultry diets, are widely consumed by humans. They are affected by interfering metabolism and negatively affect the oxidative power of yolks. Further research on several doses of these compounds and research in other animal models may be necessary to substantiate these findings, especially in the animal model of mammals.

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