

## Food Microbiology

# A comparative investigation on egg yolk total antioxidant capacity relativities to mycotoxins DON

### Abstract

DON is a member of the trichotomy family and is mainly produced by *Fusarium*. Consumption of food contaminated with DON can cause anorexia, nausea, and impaired immune function in various animals. The objective of this study is a comparative assay of egg yolk's total antioxidant capacity than to mycotoxins DON. Systems were performed by the the ELISA reader and test kit based on the kit guidances. Finally, the obtained information were analyzed with descriptive statistics and SPSS software (standard and mean deviation, contamination ratio), and a one-way analysis of variance. In the analysis of WIL COXON analysis, the mean DON values of total and P / P ((Z: -1.907 / Sig: 0.056), as well as the DON, mean of total Cub (Z: -3.660 / Sig: 0.000) were observed and the findings A similar statistical correlation can be seen between the statistical mean of total DON and the number of fatty acids measured.

**Keywords:** trichotoxin, DON, egg yolk, Mycotoxins

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### Introduction

Trichotoxins are the most important mycotoxins produced by the mevalonate pathway, which in addition to the genus *Fusarium* are also produced by other genera of fungi such as *myrtilium*, *Trichoderma*, *Trichotocium*, and *Cephalosporum*. Mycotoxins derived from cyclic polypeptides and their derivatives:

About mycotoxins derived from polycyclic peptides, we can mention Icelandic toxin, spore desmin, gliotoxin, and ergotamine. This group of toxins is produced following the addition of amino acids to micro silicon peptides or polysilicon compounds (8).

- Mycotoxins derived from amino acids and mevalonate:

Tryptophan and possibly other amino acids bind to a group of mevalonate-derived isoprene units to form amino acid and mevalonate-derived mycotoxins, producing toxins such as after and rockfortin (5).

Mycotoxins can enter the digestive system of humans and animals indirectly or directly. Indirect pollution of human and animal feed occurs when the feed is contaminated with toxin-producing fungi at some point in the production, storage, or processing process, and the mycotoxin produced will often be present in the ultimate product. pollution of grains and oilseeds is the main route for many mycotoxins to go into the animal and human food chain. Indirect pollution, food is polluted with toxin-generating fungi, and conditions are provided for the production of toxins by the fungus (12). Almost

all human and animal foods are susceptible to fungal contamination at one stage of production, processing, transport, and storage. In developed countries, the possibility of toxin spread in food is minimized by removing toxic fungi from the food chain; however, this is usually not possible in most developing countries, and moldy foods such as cereals are often an unavoidable part of the daily diet. For example, in parts of South and East Asia to Africa, the prevalence of liver cancer is associated with the persistent presence of aflatoxins and a variety of *Fusarium* toxins (10). Undoubtedly, the consumption of food contaminated with mycotoxins by animals will be associated with the presence of these toxins in various tissues as well as animal products such as milk. In practice, all components of animal and human food will be exposed to fungal contamination over some time, and in this case, the nature and extent of contamination with the toxin-producing fungus will determine the presence or absence of the product. As mentioned in the previous sections, although the identification of infectious fungi in cases of mycotoxicosis outbreaks is of diagnostic value, a definitive conclusion will be required to identify and identify potential toxins or toxins (12-14).

Therefore, the importance of mycotoxins such as DON in the food chain and human consumption is very important and in this study, we have studied it.

### Material and Methods

### **Materials in the test kit**

Each kit constructed via the Dutch company Europroxima contains the following materials:

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

0 ng / ml (standard zero), (0.313 ng / ml), (0.0625 ng / ml), (0.125 ng / ml), (0.25 ng / ml, 0.5 / ng / ml), 1 (ng / ml) of DON.

One vial of the conjugated solution is lyophilized. An antibody vial in the form of lyophilization. One vial of 12 ml substrate solution. One 15 ml stopping solution vial containing normal sulfuric acid. A 20 ml solution vial to dilute the sample and standard solution. One solution vial with a carpet volume of 30 ml.

### **Preparation of reagents**

Before starting the test, some of the ingredients fabricated and some are ready to usage. After washing, it has been concentrated 20 times and 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 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 a lyophilized kit and to utilize, 4 ml of dilution buffer should be poured to them and shaken strongly, and kept in a dark location till utilize. The chromogenic substrate solution in the kit is ready to usage and should be kept at room temperature before usage.

### **Statistical analysis method**

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

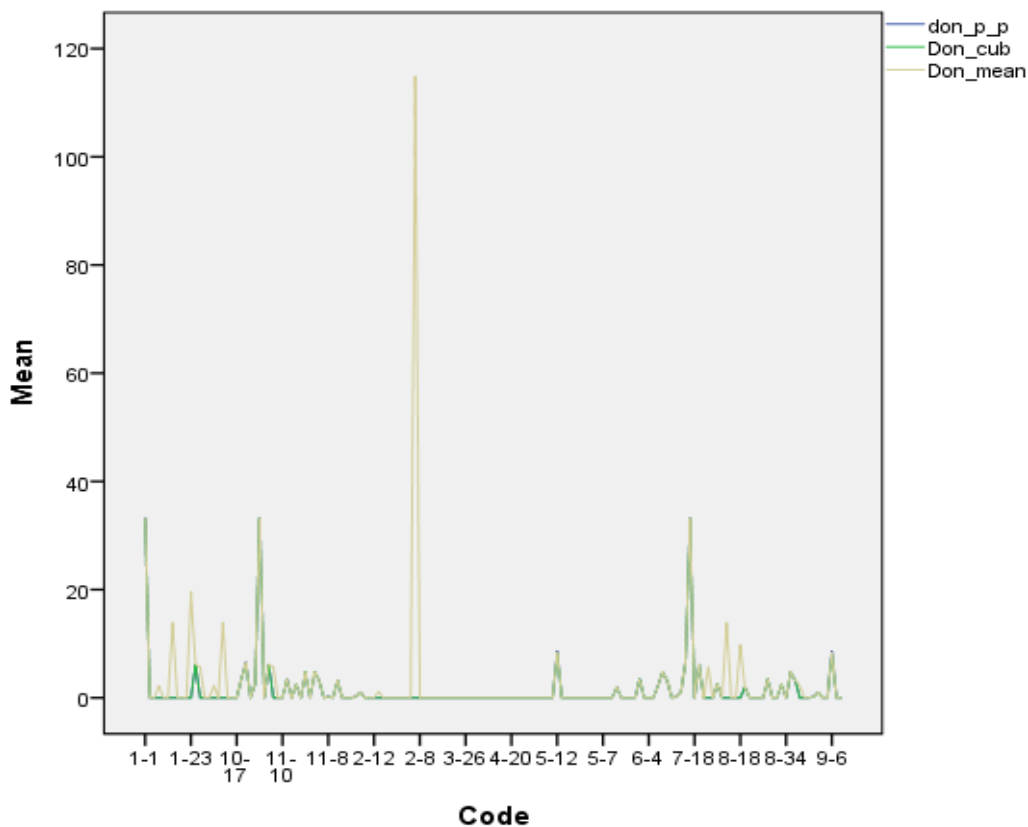
### **Results and Discussion:**

Investigation of the correlation among the measurement of Dioxinivalenone (DON) amounts and Pearson analysis

In the study of DON in all egg yolk samples, the least amount was 0.00 ppb and the utmost was 33.19 ppb in the p / p analysis also the least amount was 0.00 ppb and the utmost was 33.08 in the CUB analysis and the mean was 0.00 and utmost 114.84. The improved mean with 5% error (Trimmed mean 5%) of .5782 (P / P), .5789 (Cub), and 1.1312 demonstrated the average measured DON amounts (Figure 1-1)

The utmost DON values measured were 33.08-8.07 CUB in samples 90,122,60,118 and 1. Which include brands 7, 10, 5, 9, and 1. The lowest DON values measured were 0.00 in samples 153,150,147,145 and 154, which include brands 12,12,11,11 and 12.

**Figure 1-1:** Comparative Graphs of Mean Dioxinivalenone (DON) Values in Yolk systems obtained with the Study Brands according to Point, Point, Linear, and Cubic Function



The studies of the correlation among Pearson analysis and DON values by P / P and cub ways demonstrated a statistical correlation (PC: 1,000). This correlation amongst the average amount of total DON measured through P / P and Cub (PC: 0.415) was also statistically significant (Sig: 0.000). Hence, it can be concluded that with ignoring of the analysis and processing of amounts measured through the competitive indirect ELISA procedure, all information gained through various procedures of numerical functions in specifying the value of toxin will have the similar behavior in numerical analysis with statistical ways. This viewpoint was also observed in the analysis of WIL COXON analysis among DON values of total mean and P / P (Z: -1.907 / Sig: 0.056 and also DON mean of total Cub (Z: -3.660 / Sig: 0.000). Proves the above conclusion.

Statistical analysis of the correlation between all tested variables including the number of mycotoxins based on ppb and the amount of total antioxidant power based on nmol / g and ng / g, as well as the measured values of unsaturated fatty

acids C24 - C14 per ppm (Table 1). Two-way Pearson correlation analysis showed that the amount of aflatoxin obtained based on p / p and ochratoxin analysis as well as DON was not statistically significant. And this feature between the amounts of aflatoxin, ochratoxin, and DON mycotoxins with cub analysis also lacks any statistical correlation. So in the statistical measurement of the mean of total aflatoxin, ochratoxin and DON has not always been observed as considerable correlation. Therefore, it can be derived that the occurrence and possibility of measuring the above-mentioned mycotoxins in the egg yolk samples of the study population are not dependent on each other and act independently of each other, and therefore should be used in the prevention, tracking, and selection protocols. Evaluation, laboratory, and analysis of preliminary data and findings of statistical analysis of these mycotoxin variables should be recognized independently and researched independently of each other.

**Investigation of the correlation between the amounts of mycotoxins measured**

**Table 1:** The mean value of total DON in comparison with the fatty acids measured has a statistically significant correlation.

- a. Wilcoxon signed ranks Test
- b. Based on positive ranks





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#### **Conflict of interest**

The authors declare that there is no conflict of interest.

**Ethical statement: None**

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## **References**

1. Abdelsalam EB, el-Tayeb AE, Nor Eldin AA, Abdulmagid AM. Aflatoxicosis in fattening sheep. *Vet Rec* . 2020 ;124(18):487–8.
2. Patterson DSP, Roberts BA. Aflatoxin metabolism in duck-liver homogenates: the relative importance of reversible cyclopentenone reduction and hemiacetal formation. *Food Cosmet Toxicol*. 2021;10(4):501–12.
3. Applebaum RS, Brackett RE, Wiseman DW, Marth EH. Responses of Dairy Cows to Dietary Aflatoxin: Feed Intake and Yield, Toxin Content, and Quality of Milk of Cows Treated with Pure and Impure Aflatoxin. *J Dairy Sci*. 2019 Aug 1;65(8):1503–8.
4. ANDREW.M.HAMBLIN. A Focus on aflatoxin contamination. University of Illinois; 2019. p. 1997–2001.
5. Patterson DSP, Anderson PH. Recent aflatoxin feeding experiments in cattle. *Vet Rec* . 2020 ;110(3):60.
6. Chen W, Wang S, Zhang HX, Ruan D, Xia WG, Cui YY, et al. Optimization of dietary zinc for egg production and antioxidant capacity in Chinese egg-laying ducks fed a diet based on corn-wheat bran and soybean meal. *Poult Sci* . 2017 Jul 1;96(7):2336–43.
7. Ruan D, Fouad AM, Fan Q, Xia W, Wang S, Chen W, et al. Effects of dietary methionine on productivity, reproductive performance, antioxidant capacity, ovalbumin and antioxidant-related gene expression in laying duck breeders. *Br J Nutr* . 2018 Jan 28;119(2):121–30.
8. El-Senousey HK, Chen B, Wang JY, Atta AM, Mohamed FR, Nie QH. Effects of dietary Vitamin C, Vitamin E, and alpha-lipoic acid supplementation on the antioxidant defense system and immune-related gene expression in broilers exposed to oxidative stress by dexamethasone. *Poult Sci* . 2018 Jan 1;97(1):30–8.
9. Gardner HK, Koltun SP, Dollar FG, Rayner ET. Inactivation of aflatoxins in peanut and cottonseed meals by ammoniation. *J Am Oil Chem Soc* . 2021;48(2):70–3.
10. Samarajeewa U, Sen AC, Fernando SY, Ahmed EM, Wei CI. Inactivation of aflatoxin B1 in cornmeal, copra meal, and peanuts by chlorine gas treatment. *Food Chem Toxicol*. 2022;29(1):41–7.
11. Wang J, Yang Z, Celi P, Yan L, Ding X, Bai S, et al. Alteration of the antioxidant capacity and gut microbiota under high levels of molybdenum and green tea polyphenols in laying hens. *Antioxidants*. 2019 Oct 1 ;8(10).
12. Kullu SS, Das A, Bajpai SK, Garg AK, Yogi RK, Saini M, et al. Egg production performance, egg yolk antioxidant profile and excreta concentration of corticosterone in golden pheasants (*Chrysolophus pictus*) fed diets containing different levels of green vegetables. *J Anim Physiol Anim Nutr (Berl)*. 2017 Oct 1;101(5):e31–42.
13. Surai PF, Kochish II, Romanov MN, Griffin DK. Nutritional modulation of the antioxidant capacities in poultry: the case of vitamin E. *Poult Sci* . 2019 Sep 1;98(9):4030–41.
14. Hargitai R, Nyiri Z, Eke Z, Török J. Effects of temperature and duration of storage on the stability of antioxidant compounds in egg yolk and plasma. *Physiol Biochem Zool*. 2018 Mar 1;89(2):161–7.
15. Reis JH, Gebert RR, Barreta M, Boiogo MM, Souza CF, Baldissera MD, et al. Addition of grape pomace flour in the diet on laying hens in heat stress: Impacts on health and performance as well as the fatty acid profile and total antioxidant capacity in the egg. *J Therm Biol*. 2019 Feb 1;80:141–9.

16. Surai PF. Antioxidants in poultry nutrition and reproduction: An update. Vol. 9, *Antioxidants*. MDPI AG; 2020.

17. Dong XF, Zhai QH, Tong JM. Dietary choline supplementation regulated lipid profiles of egg yolk, blood, and liver and improved hepatic redox status in laying hens. *Poult Sci* . 2019 Aug 1;98(8):3304–12.

18. Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant defense systems and oxidative stress in poultry biology: An update. Vol. 8, *Antioxidants*. MDPI AG; 2019.