

Microbiological and Toxicological Evaluation of Broiler and Domestic Chickens from Local Markets of Peshawar, Pakistan

Monib Ullah^{1*}, Hikmat Ullah¹, Khyber² and Atifullah Shinwari³

¹Center of Biotechnology and Microbiology, University of Peshawar, Pakistan

²Institute of Microbiology, University of Agriculture Faisalabad, Punjab, Pakistan

³Department of Horticulture, University of Agriculture, Peshawar, Pakistan

*Corresponding Author: Monib Ullah, Center of Biotechnology and Microbiology, University of Peshawar, Pakistan.

Received: December 15, 2019; Published: February 25, 2020

Abstract

Mycotoxins are secondary metabolites, important due to their toxic effects in humans and animals. Undesirable consequences are often resulted if contaminated food or feed is consumed by human and animals in enough quantity. In this study, samples of commercial broiler and domestic chickens were obtained and analyzed for microbial and mycotoxin contamination. Microbiological testing of domestic chickens revealed that these samples were only contaminated with bacterial species while no fungal growth was observed. In contrast, the broiler chicken samples were contaminated with bacteria as well as fungi. Furthermore, in toxicological analysis, peaks of different toxins were obtained by HPLC with PDA detector at wavelength of 322 nm to 340 nm in a long program of 50 minutes of time. Ochratoxin-A had noticed at 11.0 minutes of retention time, while Penicillic acid had discovered at 3.45 minutes of retention time. The investigation showed that both types of meat samples were contaminated with toxins. Since both samples were obtained from the same geographical area and consumed the same feed, hence, these results suggest that the feed consumed by the two types of chickens was of the same type and actually from the same source of toxins.

Keywords: Chicken Meat Samples; Standard Toxins; Mycotoxins Analysis; HPLC; Bacterial Analysis; Bacterial Cultures

Introduction

Mycotoxins are low molecular weight substances which exert it toxic effect on the health of animals, humans, and plants [1] mostly effects occur when contaminated food is consumed for long time or in higher quantity. Approximately 200 species of filamentous fungi are potent producers of mycotoxins [2] of nearly 400 diverse types including the Aflatoxin the most famous one for health consequences [3]. More than one mycotoxins are produced by a single cell while more than one species can produce the same mycotoxin [4]. It is convenient to say that mycotoxins have two crucial factors in causing disease, the target and concentration, while it is inconvenient to say all the toxic compounds produced by fungi termed as mycotoxins [1,5]. The classification of mycotoxins is based on organ it affects, biosynthetic origin, generic group, chemical structure, disease it cause and productive organism. Mycotoxins can be teratogens, carcinogens, mutagens and allergens as well [1]. Fungi produces 31,000 different secondary metabolites which are not necessarily mycotoxins at all but contains a few number of mycotoxins [6]. But fortunately a fraction of them are hazardous to humans and animals [7].

About 25% of agricultural cereals are contaminated with mycotoxins [8]. Those fungal secondary metabolites which can elicit a deleterious effect on humans, plants and animals health are termed mycotoxins [9]. Wheat contamination with mycotoxins is 25% on worldwide

level [9]. Some types of mycotoxins synthesized by microorganism confer harmful effect on other microbes like penicillin produced by *Penicillium chrysogenum* inhibit the synthesis of peptidoglycans in bacteria [10]. Edible food products are the only exposure components which causes mycotoxicosis in humans but recently airborne mycotoxins has elucidated [11]. Moisture, time and temperature are basic factors in determining the production of mycotoxins by the organism [12]. The relative temperature for mycotoxins synthesis is between 4 up to 32 degree centigrade while the moisture content should be 22 to 23% in grains, 1 to 2% oxygen is required in the presence of more than 70% humidity [1]. Mycotoxins confer resistance to heat and temperature, they cannot be numbed due to temperature treatments [13,14]. Ochratoxin-A is resistant from 100 up to 250 degree centigrade in different moisture condition so it require a more sophisticated and efficient dogma for decontamination [15]. Zearalenones, Aflatoxins, ochratoxins and deoxynivalenol are commonly famous for their bad impact on humans and animals [16]. An epidemiology with Aflatoxins in England (1960) where approximately 100,000 turkeys were taken under the effect and were expired [18]. 4.5 billion of entire world population is exposed to the risk of Aflatoxin stated by Wild and Gong [18].

Materials and Methods

Research area

The entire research work has been conducted in two direction for-example, the analysis of microbes and toxins. To examine microbe presence in the samples, microbiology and biotechnology department of the University was preferred. The toxins analysis was accomplished at the department of agriculture chemistry, University of Agriculture Peshawar.

Sample size and location

There were 10 chickens tested in this study, 5 chickens from GT road Gulabad near to Saokano mor on 13 April 2016 at 8:00 PM evening. The other chickens were taken from Hashtnagri Peshawar from local chicken market on 13 April 2016 at 6:00 PM. The third one domestic chicken was taken from chargano chaok on 20th April at 4:00 PM. There were 5 commercial broilers and 5 domestic chickens.

Microbiological analysis

Blood samples were collected in EDTA tube in order to avoid coagulation. The meat samples were cut with sterile knife and kept in air-tight bags. All the samples were brought to microbiology lab of center of microbiology and biotechnology department. From each meat sample, one gram was weighed aseptically with an electronic balance. It was macerated by blending and dispersed in 9 ml of sterile distilled water in a sterile test tube, further 10 micro litre sample was taken, the suspension was placed in the center of the plate using a sterile pipette and spread onto the petri plates containing growth media by spread plate method in LFH. The samples were cultured on four different types of media i.e. Mackonkey agar, nutrient agar, mannitol salt agar and Czapek yeast extract agar media. Nutrient agar media along with Mackonkey agar, Mannitol salt agar, were utilized for bacterial growth whereas, CYA media were used for fungal growth. Inoculation was done by spread plate method. After the incubation, the plates were observed for morphology, gram staining and biochemical tests.

Method of toxicological analysis

The toxicological analysis was done only for the detection of Ochratoxin A. 25g of each chicken samples was grinded and mixed with methanol to extract the metabolites from the sample. Chloroform and methanol both used as extraction solvents. All the samples kept in glass jars for several hours after shaking with 10 minutes of time. 11 micrometer pore size filter paper was used to filter the solution so that to get the extract from each sample. Next a separator funnel used to remove the water layers from the filtrate. The resulted filtrate now poured into round bottom flask for rotary evaporation to acquire solvent from the solution under vacuum. Next, I added 3 ml of HPLC grade methanol again to each sample extract and the extract transferred to supplied vials. Now further each sample was filtered with the help of nylon syringe filter having pore size of 0.44 micrometer. The final filtrate was now poured into individual HPLC glass vials having size of 1.5 ml.

Stock solution synthesis for Ochratoxin-A

Stock solution = 1 mg OTA/ml of methanol

Or

1000 (1000 ppm) of OTA/ml of methanol

With the help of a plastic syringe, 1 ml of HPLC grade methanol added to the glass bottle holding 1 mg of OTA. From this standard solution further dilutions of the standard OTA were synthesized.

Preparation of dilutions

From the stock solution, dilutions were made for standard curve preparation. Seven different dilutions were prepared with the following concentration. $C1V1 = C2V2$ is a formula used for the formation of seven different dilutions. Following are the concentrations of dilutions.

C-7	10 ppm
C-6	5.0 ppm
C-5	1.0 ppm
C-4	0.4 ppm
C-3	0.2 ppm
C-2	0.1 ppm
C-1	0 ppm

Table: Concentrations of dilutions prepared from stock solutions.

Chromatographic analysis

Binary pump HPLC apparatus with a photo diode array (PDA) detector was used in this research investigation. Before the sample insertion, the apparatus was washed with 50% water and 50% methanol to remove any contaminant if present. After washing, the standard toxins dilutions were injected to HPLC machine for running to make standard curve which then compares with our desired sample toxin. 20 micro liter of liquid sample was then injected into HPLC machine by using an HPLC syringe for each sample.

Below are the pumps for which gradient was set using the HPLC software.

Time range (in minutes)	First pump-A	Second Pump-B
0 - 5	95	5
5 - 20	30	70
20 - 30	20 - 30	70 - 80
30 - 35	10 - 20	80 - 90
35 - 43	5	95
43 - 50	95	5

Results and Discussion

Microbiological examination of domestic chickens

All the 5 domestic chickens' samples were subjected to culture on different media. Three samples from each chickens were subjected including croup and flesh. There were only 4 chicken croups samples which were contaminated with bacteria while no any fungal growth

had occurred. In case of meat sample, only one chicken meat sample showed to be contaminated with bacteria. The figure below shows bacterial count in CFU/g (CFU/gram).

Sample type	First-1	Second-2	Third-3	Fourth-4	Fifth-5
Croup	1.53 x 10 ⁵	0	4.7 x 10 ⁴	1.20 x 10 ⁶	6.3 x 10 ⁴
Meat	0	1.8 x 10 ⁴	0	0	0

Microbiological examination of broiler chickens

Out of 5 broiler chickens, the croup sample of 4 chickens were contaminated with bacteria while there was no growth of fungi observed, but the meat samples of broiler chickens were contaminated with different species of bacteria as well as fungi. The figure below shows the microbial count of bacteria in CFU/g.

Sample type	First-1	Second-2	Third-3	Fourth-4	Fifth-5
Croup	0	3.1 x 10 ⁶	4.32 x 10 ⁶	1.6 x 10 ⁵	4.0 x 10 ⁵
Meat	0	2.13 x 10 ⁶	1.9 x 10 ⁶	6.0 x 10 ³	4.7 x 10 ¹

The blood showed no contamination with either bacteria or fungi from both types of chickens, so blood was sterile.

Toxicological evaluation

Only the meat samples of the domestic and broiler chickens evaluated through HPLC for the presence of Fungal toxins. Different magnitudes of the results occurred. The concentration of different toxins were obtained using HPLC photodiode detector along with some other toxins OTA and PA were observed. OTA was resolved at 11.0 minutes while PA was resolved in 3 - 5 minutes.

Standard peak of OTA

OTA standard was run on HPLC in a 50 minute program. On 5 parts per million the OTA standard showed peak at retention time 11.012 minutes shown in figure 1 and at 10ppm the holding time for OTA was noticed to be 11.059 shown below figure 2.

The graphs below are taken from HPLC system.

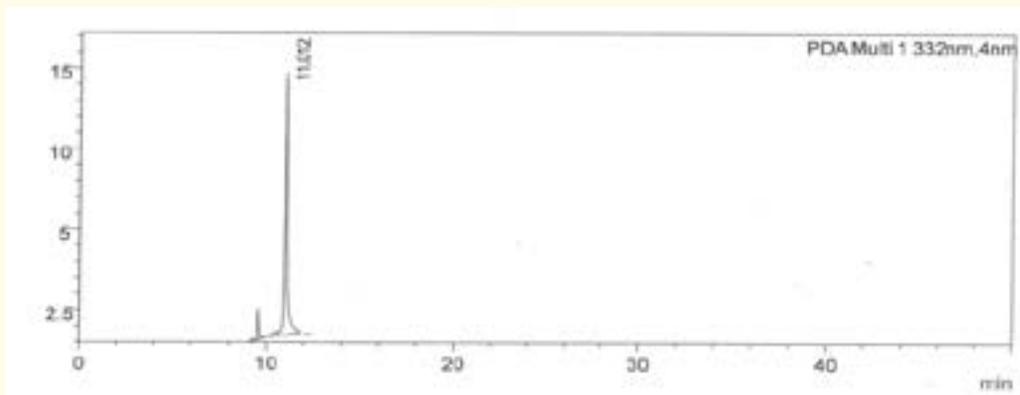


Figure 1: Chromatogram of standard OTA detection at 5ppm which shows a peak at retention time of 11.012 minutes.

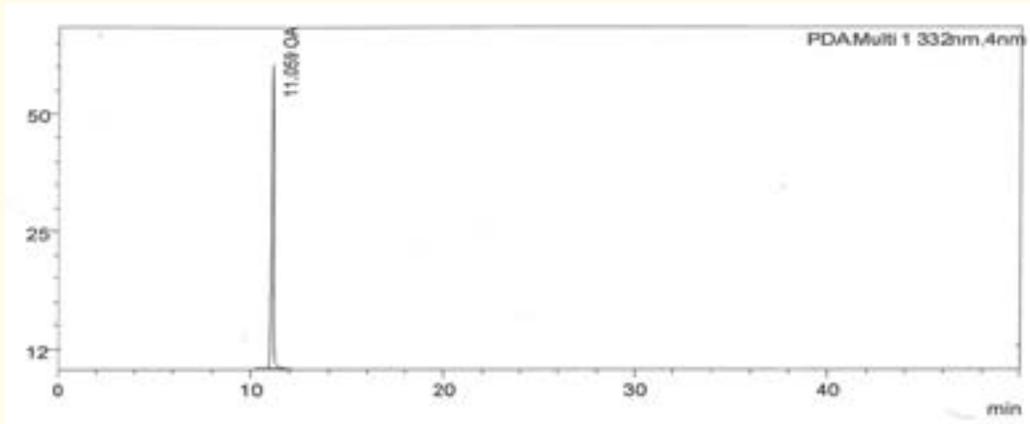


Figure 2: Chromatogram of standard OTA detection at 10ppm which shows a peak at retention time of 11.059 minutes.

Toxicological evaluation of domestic chickens

The toxicological evaluation of domestic chicken show many peaks at different retention time along with the peak at retention time of 11.029 minutes which is for OTA and peak at 3.45 minutes which is retention time for penicillic acid as shown in below figure 3.

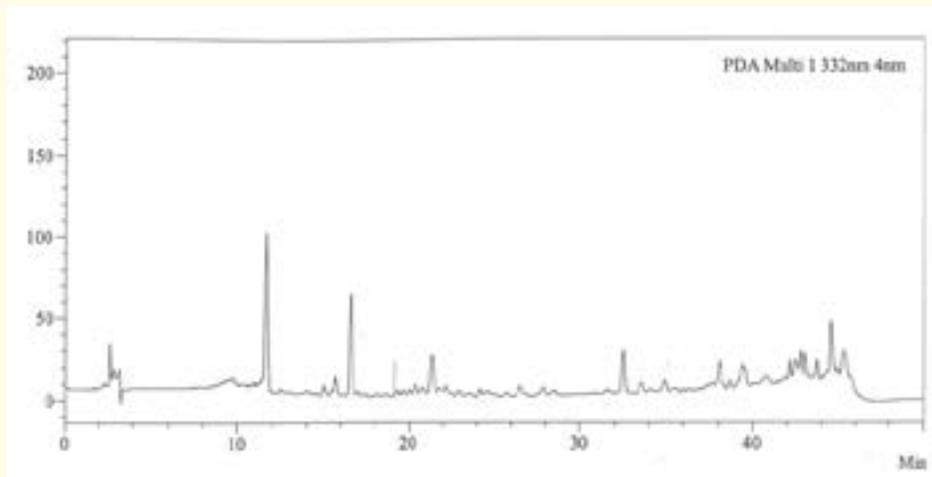


Figure 3: Chromatogram of domestic chicken meat extract, which shows peaks at different retention times including peaks at retention time of 11.029 minutes, which is for OTA and at 3.45 minutes, which shows the retention time for PA.

Toxicological evaluation of broiler chickens

The toxicological evaluation of broiler chicken show many peaks at different retention time along with the peak at retention time of 11.023 minutes which shows OTA and peak at 3.45 minutes which is retention time for Penicillic acid as shown in the figure 4.

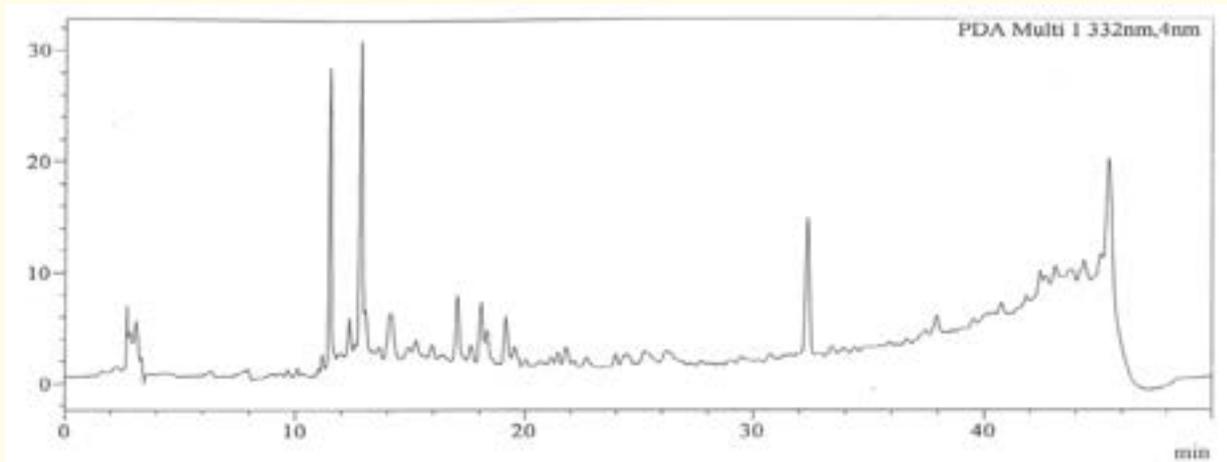


Figure 4: Chromatogram of broiler chicken meat extract which shows peaks at different retention times including peaks at retention time of 11.023 minutes, which is for OTA and at 3.5 minutes which shows the retention time for PA.

Conclusion

Microbiological and toxicological evaluation of the two types of chickens, the broiler and the domestic, revealed that they both were contaminated with microorganisms and mycotoxins. This result suggests that since both the samples were obtained from the same geographical area, they have been given the same feed which ultimately resulted in their microbial and toxicological contamination. If such contaminated chickens are consumed on a regular base, so most probably there can be sufficient chance of contracting a health condition in humans. Considering these evidences in account, I recommend that proper methods for quality control of chicken feed must be generated and applied to avoid health hazards in chickens as well as humans. Feed manufacturers must regularly examine their feed for microbial and toxicological contamination to ensure that the available feed is healthy and hygienic for consumption by primary and secondary consumers.

Bibliography

1. Gulyás N. "Mycotoxin contamination in food-and feedstuffs" (2013).
2. Abdallah MF, *et al.* "Occurrence, prevention and limitation of mycotoxins in feeds". *Animal Nutrition and Feed Technology* 15 (2015): 471-490.
3. Chen MT, *et al.* "Mycotoxin monitoring for commercial foodstuffs in Taiwan". *Journal of Food and Drug Analysis* 24.1 (2016): 147-156.
4. Hussein HS, *et al.* "Toxicity, metabolism, and impact of mycotoxins on humans and animals". *Toxicology* 167.2 (2001): 101-134.
5. Zain ME. "Impact of mycotoxins on humans and animals". *Journal of Saudi Chemical Society* 15.2 (2011): 129-144.
6. Berthiller, *et al.* "Determination of a Deoxynivalenol Glucoside in Artificially and Naturally Contaminated Wheat by Liquid Chromatography-Tandem Mass Spectrometry". *Journal of Agricultural and Food Chemistry* 53.9 (2005): 3421-3425.

7. Berthiller F, *et al.* "Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals". *International Journal of Food Microbiology* 119.1 (2007): 33-37.
8. Marin S., *et al.* "Mycotoxins: occurrence, toxicology, and exposure assessment". *Food and Chemical Toxicology* 60 (2013): 218-237.
9. Abbott SP. "Mycotoxins and indoor molds". *Indoor Environment Connections* 3.4 (2002): 14-24.
10. Griffith DP. "Struvite stones". *Kidney International* 13.5 (1978): 372-382.
11. Dall AC. "Mycotoxins and Nuclear Receptors: A Still Underexplored Issue". *Nuclear Receptor Research* 3 (2016).
12. Anukul N., *et al.* "Significance of regulation limits in mycotoxin contamination in Asia and risk management programs at the national level". *Journal of Food and Drug Analysis* 21.3 (2013): 227-241.
13. De Ruyck K., *et al.* "Dietary mycotoxins, co-exposure, and carcinogenesis in humans: Short review". *Mutation Research Reviews in Mutation Research* 766 (2015): 32-41.
14. De Boevre M., *et al.* "Metabolism of modified mycotoxins studied through invitro and in vivo models: An overview". *Toxicology Letters* 233.1 (2015): 24-28.
15. Boudra H., *et al.* "Thermostability of Ochratoxin A in wheat under two moisture conditions". *Applied and Environmental Microbiology* 61.3 (1995): 1156-1158.
16. Li X., *et al.* "Occurrence of mycotoxins in feed ingredients and complete feeds obtained from the Beijing region of China". *Journal of Animal Science and Biotechnology* 5.1 (2014): 37.
17. Stoev S D. "Foodborne mycotoxicoses, risk assessment and underestimated hazard of masked mycotoxins and joint mycotoxin effects or interaction". *Environmental Toxicology and Pharmacology* 39.2 (2015): 794-809.
18. Wild CP, *et al.* "Mycotoxins and human disease: a largely ignored global health issue". *Carcinogenesis* 31.1 (2009): 71-82.

Volume 6 Issue 3 March 2020

©All rights reserved by Monib Ullah., *et al.*