



Aflatoxins B1 and B2 residues in Edible Offals

Ibrahim H.M., Hassan M. A. and Elsharkawy G. S.

Department of Food Hygiene. Faculty of Veterinary. Medicine. Benha University.

ABSTRACT

In kalyobia governorate a total of 90 random samples of chicken giblets represented by liver, gizzard and heart (30 of each) were purchased from different poultry shops for determination of their concentrations of serious aflatoxins and their validity for human consumption. The obtained results revealed that aflatoxins B1 was detected in liver, gizzard and heart samples at 26.67, 23.33 and 10 % respectively with mean concentration of $27.03 \pm 3.65 \mu\text{g/kg}$, $18.54 \pm 3.65 \mu\text{g/kg}$ and $7.98 \pm 1.10 \mu\text{g/kg}$ of AFB1, respectively. AFB2 was detected in liver, gizzard and heart samples at 20, 13.33 and 6.67 % with mean concentration of $13.62 \pm 1.94 \mu\text{g/kg}$, $9.94 \pm 1.25 \mu\text{g/kg}$ and $4.79 \pm 0.81 \mu\text{g/kg}$ of AFB2, respectively, with high significant differences between examined giblets. Liver samples showed the highest levels of AFB1 and AFB2, followed by gizzard samples, while the heart samples recorded the lower levels in comparison with previous two giblets.

Key words: AFB1, AFB2, edible offals.

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1. INTRODUCTION

Chicken meat and chicken meat products are not only tasteful, economical, quick and easy to prepare food but also provide a unique well balanced source of minerals, vitamins, proteins and healthy fats for all ages. Moreover, their high quality, low caloric value and ease to digestibility make chicken valuable in many therapeutic diets for adults. AF contamination is still a threat to the poultry industry and results in substantial economic losses to producers because of often sub-lethal, but toxic, effects. Hepatocellular carcinoma (HCC) is one of the leading primarily affecting populations in the developing countries. Aflatoxin a food contaminant is a known human carcinogen that has been shown to be a causative agent in

the pathogenesis of HCC. (Magnussem and Parsi, 2013). In addition, Felicia et al., 2014, mentioned that aflatoxins cause liver cancer and have also been implicated in child growth impairment and acute toxicoses; fumonisins, which have been associated with esophageal cancer (EC) and neural tube defects (NTDs); deoxynivalenol (DON) and other trichothecenes, which are immunotoxic and cause gastroenteritis.

The present study is planned to throw light on aflatoxin residues in some chicken edible offals in some Qalyobia governorate markets.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Collection of samples:

A total of 90 random samples of chicken giblets represented by liver, gizzard and heart (30 of each) were purchased from different poultry shops located in Kalyobia governorate. Each sample was kept in a sterile plastic bag and preserved in an ice box as well as transferred to the laboratory under complete aseptic conditions without undue delay. All collected samples were mycologically examined as quickly as possible for determination of their concentrations of aflatoxins B₁ and B₂ and their validity for human consumption.

2.2. Methods:

2.2.1. Qualitative and quantitative estimation of aflatoxins:

2.2.1.1. Preparation of chemicals:

Standard and Blank aflatoxins B₁ and B₂ were diluted in benzene: acetonitrile of chromatographic grade. The methanol, HPLC grade, used for the preparation of the mobile phase and elution of aflatoxin in the immunoaffinity column.

Column storage took place at a temperature ranging from 2 and 8° C and they were used at room temperature. The entire glassware used for aflatoxin determination was decontaminated by Alkaline Extran MA 01, 7555 20%, (pH > 12), remaining in contact for 24 hours and further washing with distilled water.

2.2.1.2. Standard Aflatoxin solutions:

The stock standard solutions of B₁ and B₂ were prepared by dissolving the solid standard in benzene: acetonitrile (98:2, v/v). The precise concentration was measured in

Shimadzu UV-1601 PC spectrophotometer, Shimadzu Scientific Instruments, Japan, as described by AOAC (2000).

An intermediate standard solution from the stock solution was prepared in benzene: acetonitrile (98: 2, v/v) in a concentration of 9.855 ng ml⁻¹. This solution was utilized for the elaboration of a calibration curve in the range 0.1–9.8 ng/ml. All the solutions were packed in the amber vials at -18°C.

2.2.1.3. Extraction and clean-up procedures for high-performance liquid chromatography (HPLC) analysis:

The prepared samples were analyzed using a validated method by reversed-phase HPLC separation and fluorescence detection after post-column derivatization (Shundo and Sabino, 2006).

2.2.1.4. Determination of aflatoxins by HPLC method:

The presence of aflatoxins B₁ and B₂ detected by HPLC after post-column derivatization with the electrochemical generation of bromine (KOBRA cell – Rhone diagnostic technologies, UK) with a current of 100 µA and a fluorescence detector (Shimadzu LC-10 AD Model; 360 nm excitation wavelength; 435 nm emission wavelength; with Shim-Pack CLC – ODS column, 5 µm, 4.6 × 250 mm, preceded by a guard column Shim – Pack G – ODS, 5 µm, 4 × 10 mm). The mobile phase was deionized water-acetonitrile-methanol (60:20:20, v/v/v) with the addition of 350 µL of 4M HNO₃ and 120 mg of KBr at a flow rate of 1 ml/min. The injection volume was 50 µl. The quantification of aflatoxin was performed by measuring its peak areas at each retention time and comparing it with the calibration curve (Galvano et al., 2001). The performance of the method, aflatoxin recovery and

effectiveness of the cleanup procedure, was evaluated by the samples spiked with this aflatoxin.

2.2.2. Statistical analysis:

The obtained results were statistically analyzed by application of Analysis of variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

3-1: Aflatoxin B1 residues in some chicken edible offals:

4-1-1. Incidence of aflatoxin B1 in the examined samples of chicken giblets was presents in Table, 1 and figure, 1

It is obvious that the presence of aflatoxin B1 was detected in 26.67% of liver samples (8 samples), while, 23.33% of Gizzard samples (7 samples) showed incidence of aflatoxin B1, whereas heart samples recorded the lowest percentage of aflatoxin B1 incidence (10%; i.e. 3 samples).

3-1-2. Aflatoxin B1 levels ($\mu\text{g/Kg}$) in the examined samples of chicken giblets are tabulated in Table, 2 and figure, 2.

Results declared that mean of aflatoxin B1 concentrations in liver samples was the higher mean (27.03 ± 3.65) in comparison to the other two giblets (i.e, gizzard and heart), followed by the mean of gizzard samples (18.54 ± 2.42), while mean of heart samples came at last with mean of (7.98 ± 1.10).

Analysis of variance (ANOVA) of aflatoxin B1 levels in the examined samples of chicken giblets are posted in Table, 3.

Obtained results shown in Table, 3 clearly showed that there are a high

significant differences between examined giblets with 7.24 ++ ($p < .01$).

3-2: Aflatoxin B2 residues in some chicken edible offals:

Incidence of aflatoxin B2 in the examined samples of chicken giblets; Statistical analytical results of aflatoxin B2 levels ($\mu\text{g/Kg}$) in in the examined samples of chicken giblets and Analysis of variance (ANOVA) of aflatoxin B2 levels in the examined samples of chicken giblets are shown in Table, 4 and figure, 3; Table, 5 and figure, 4 and Table, 6 respectively.

4-2-1. Data presents in Table, 4 and figure, 3 indicated that 20% of liver samples (6 samples) recorded incidence of aflatoxin B2, whereas, 13.33% of gizzard samples (4 samples) showed presence of aflatoxin B2. Besides, 6.67% of heart samples (2 samples) were incidence of aflatoxin B2.

4-2-2. Statistical analytical results of aflatoxin B2 levels ($\mu\text{g/Kg}$) in in the examined samples of chicken giblets are tabulated in Table 5 and figure, 4. It is obvious that liver samples had the highest concentration of aflatoxin B2 (13.62 ± 1.94) in comparison with the other two giblets (gizzard and heart) which recorded 9.94 ± 1.25 and $4.79 \pm .81$ respectively.

4-2-3. High significant differences were obtained between examined giblets 5.09 ++ ($p < 0.01$) through analysis of variance (ANOVA) of aflatoxin B2 levels in the examined samples of chicken giblets (Table, 6).

Table (1): Incidence of aflatoxin B1 in the examined samples of chicken giblets (n=30).

Chicken giblets	%
Liver	26.67
Gizzard	23.33
Heart	10
Total (90)	20

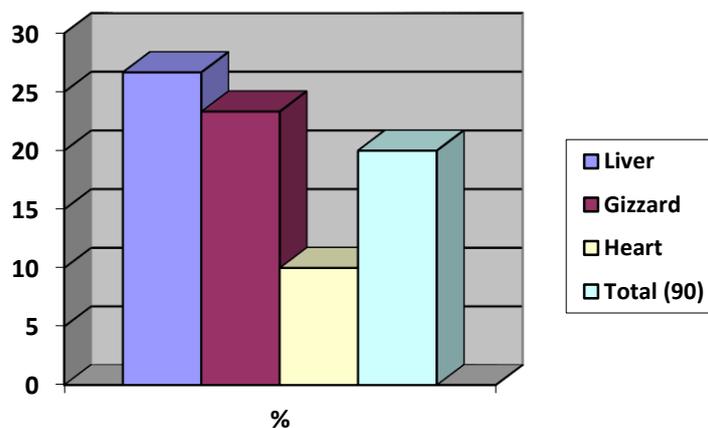


Figure (1): Incidence of aflatoxin B1 in the examined chicken giblets.

Table (2): Statistical analytical results of B1 levels aflatoxin (ug/Kg) in the examined samples of chicken giblets (n=30).

Chicken giblets	Mean
Liver	27.03
Gizzard	18.54
Heart	7.98

S.E* = standard error of mean Mean value was calculated according to positive samples

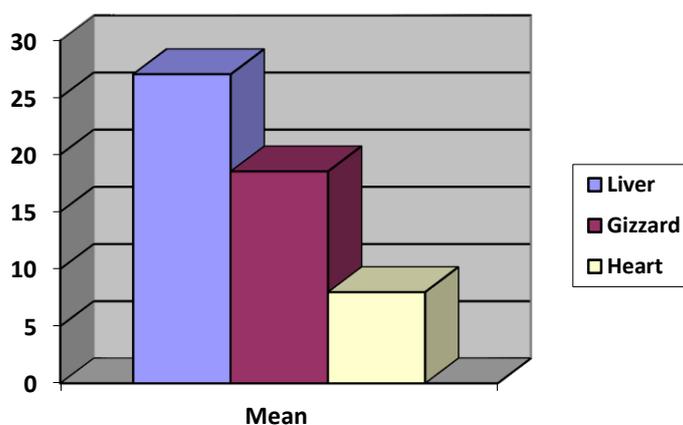


Figure (2): Average concentrations of aflatoxin B1 (µg/Kg) in the examined chicken giblets.

Table (3): Analysis of variance (ANOVA) of aflatoxin B1 levels in the examined samples of chicken giblets.

Source of variance	D.F	S.S	M.S	F.value
Total	89	16627.58		
Between Giblets (G)	2	2372.61	1186.30	7.24 ⁺⁺
Error	87	14254.97	163.85	

D.F = Degrees of freedom

S.S = Sum squares

M.S = Mean squares

++ = High significant differences (P<0.01)

Table (4): Incidence of aflatoxin B2in the examined samples of chicken giblets (n=30).

Chicken giblets	%
Liver	20
Gizzard	13.33
Heart	6.67
Total (90)	13.33

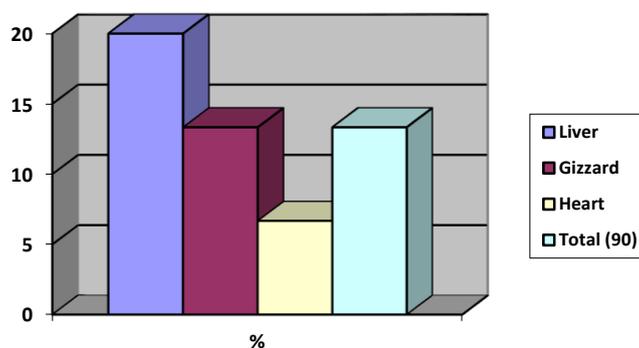


Figure (3): Incidence of aflatoxin B2in the examined chicken giblets.

Table (5): Statistical analytical results of aflatoxin B2 levels (ug/Kg)in the examined samples of chicken giblets (n=30).

Chicken giblets	Mean
Liver	13.62
Gizzard	9.94
Heart	4.79

S.E* = standard error of mean

Mean value was calculated according to positive sample

Table (6): Analysis of variance (ANOVA) of aflatoxin B2 levels in the examined samples of chicken giblets.

Source of variance	D.F	S.S	M.S	F.value
Total	89	9162.02		
Between Giblets (G)	2	959.67	479.83	5.09 ⁺⁺
Error	87	8202.35	94.28	

D.F = Degrees of freedom S.S = Sum squares M.S = Mean squares

++ = High significant differences (P<0.01)

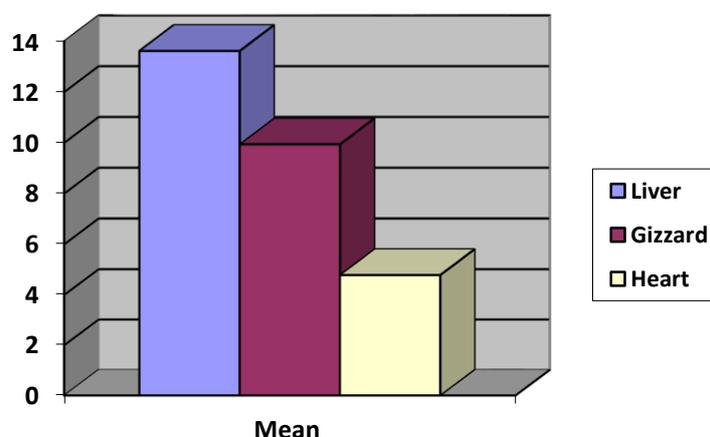


Figure (4): Average concentrations of aflatoxin B2 (µg/Kg) in the examined chicken giblets

4. DISCUSSION

Incidence of aflatoxins in chicken offals was previously reported by many authors; Khan et al., 2013; Al-Ameiri et al., 2014 and El-Desouky et al., 2014. They detected aflatoxins in chicken offals (liver, gizzard and heart) at different percentages and at different concentrations.

The obtained results of aflatoxin B1 came in line with those found by El-Desouky et al., 2014 who found that the percentages of AFB1 contaminated samples of liver were the highest percentage, followed by Gizzard samples, followed by heart samples (45, 32 and 25 respectively). In addition, Sineque et al, 2017 found that poultry liver samples recorded

AFB1 percentage above gizzard samples (39 % and 13.8% respectively). Besides, Iqbalet al. , 2014 found that 35% of chicken meat samples were contaminated with aflatoxins, they added that the highest concentration of AFB1 and total aflatoxins detected in livers (2.98 ± 0.76 and $3.23 \pm 0.82 \mu\text{g} / \text{Kg}$, respectively). In addition, they explained the presents of aflatoxin B1 in high percentages and high levels in liver samples that feeding and feed managements can be main source of aflatoxins.

The obtained results of AFB2 came in harmony with those obtained by El-Kewaiey et al., 2010; they found in chicken meat samples that the 5th one has $0.4 \mu\text{g} / \text{g}$ AFB2. Besides, two of duck meat samples were

positive to aflatoxins; the first one has 0.5 µg / Kg of AFB₂. These results recorded lower values of AFB₂ in comparison with the obtained results in present study (13.62 ±1.94 µg / Kg; 9.94 ±1.25 µg / Kg; 4.79 ±0.81 µg / Kg for liver, gizzard and heart respectively). On the other hand, Shaltout et al, 2014 detected AFB₂ in some meat products (Kofta, 8.5 ±1.07µg / Kg; Sausage, 5.20 ±0.69 µg / Kg; Lunchon, 5.17 ±0.72 µg / Kg and Basterma, 2.33 ± 0.15 µg /Kg). The above mentioned results came also in lower concentrations than the obtained results of chicken offal, i.e., liver, gizzard and heart (13.62 ±1.94 µg / Kg; 9.94 ±1.25 µg / Kg; 4.79 ±0.81 µg / Kg respectively)

Regarding present study results, the edible chicken offals (i.e. liver, gizzard and heart) samples were contaminated with higher concentrations of aflatoxin B₁, B₂, G₁ and G₂ than the permissible limits of both United States Food and Drug Administration (20ppb in food for human conception.) and European Union permissible limits of AFB₁ and total aflatoxin (2 and 4 µg/kg) with highly differences .This present the seriousness of probability incidence of above mentioned risks caused by intake contaminated edible chicken offals with aflatoxins.

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