Aflatoxin B_1 residues in imported and local broiler's breast and thigh muscle in Kurdistan region

E.P. Candlan[1], I.Q. Sito[1], M.Y. Yalda[1], S.N. Taheer[1] and A.M. Shareef[2]

[1] Central Veterinary Laboratory, Erbil, Kurdistan, [2]Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, E-mail: aqeel.shareef@yahoo.com

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Abstract

Residues of Aflatoxins and their metabolites might be present in meat and other products of animals receiving Aflatoxin contaminated feeds which could subsequently create health problems in man. Eighty nine imported (Iran/Khosh pokht); (Turkey/Yam-tapilic, Lades, Senplic, Kapidac, Kozoa, Oznesilpilic) and (Brazil, hilal, Sadia), and 90 locally produced (Hoshiar poultry farm, Nihad poultry farm, Hokar poultry farm, Mansoor poultry farm, AL-Shimal poultry house, Mardin poultry house and AL-Eetimad poultry slaughterhouse) broiler breast and thigh muscle samples were examined for residual Aflatoxin B_1 using ELIZA test. Results revealed that out of 89 imported samples only 21 (23.59%) were positive, but only 2 (2.24%) were rejected, while the remaining 87 samples (97.75%) were acceptable. Concerning the local samples, results showed that 19 samples (21.11%) were positive, but 10 (11.11%) were rejected, while the remaining 80 samples (88.88%) were accepted. The public health importance of residual AFB_1 in broiler meat samples was discussed.

Keywords: Aflatoxin, Broiler meat, ELISA
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بقايا سم الافلا B_1 في لحوم الافخاذ والصدر لفروج اللحم المحلي والمستورد في اقليم كوردستان

الهام بطرس شابو كندلان[١]، انعام قاسم سيتو[١]، مارتا يونان يلدا[١]، سامان نجيب محمد طاهر[١]، عقيل محمد شريف[٢]

[١] المختبر البيطري المركزي، اربيل، كوردستان، [٢] فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

قد تتواجد بقايا سم الافلا ومئيضاته في لحوم الحيوانات ومنتجات الحيوانات المستهلكة لاعلاف ملوثة بسموم الافلا والتي قد تسبب في حدوث مشاكل صحية لدى المستهلك. جمعت تسع وثمانون عينة من عينات فروج اللحم المستوردة (ايران، Khosh pokht) و (تركيا، Yam-tapilic, Lades, Senplic, Kapidac, Kozoa, Oznesilpilic) و (البرازيل، hilal, Sadia) وكذلك تسعون عينة من لحوم الافخاذ والصدر المحلية (الماخوذة من حقول دواجن هوشيار ونهاد و هوكر ومنصور والشمال وماردين ومجزرة الاعتماد) وذلك للكشف عن تواجد سموم الافلا اومئيضاتها من سم الافلا B_1 باستخدام تقنية الادمصاص المناعي المرتبط بالانزيم (ELISA). كشفت النتائج أنه من أصل ٨٩ عينة من العينات المستوردة ٢١ (٢٣,٥٩ ٪) عينة موجبة، الا ان عينتين فقط من العينات الموجبة (٢,٢٤٪) رفضت وقبلت العينات الـ ٨٩ عينة (٩٧,٧٥٪). اما العينات المحلية فقد أظهرت النتائج أنه ١٩ (٢١,١١٪) عينة كانت موجبة الا ان عشر عينات (١١,١١٪) رفضت وقبلت العينات الـ ٨٠ الباقية (٨٨,٨٨٪). نوقشت الأهمية الصحية العامة لبقيا AFB_1 في عينات لحوم فروج اللحم.

Introduction

Hepatocarcinogicity of Aflatoxins has been observed in several experimental animals and humans because of ingestion of Aflatoxin contaminated foods in areas of Asia, USA, the European community and Africa by epidemiological studies (1). A large number of observations have shown that hepatic metabolism plays a prominent role in determining the biological action of Aflatoxin B_1 (AFB_1). Conversions of Aflatoxin B_1 to Aflatoxin M_1,

Aflatoxin P_1, Aflatoxin Q_1, Aflatoxin B_2 and Aflatoxicol are regarded to be detoxification steps, because the toxicity or carcinogicity of these metabolites are lower than those of AFB_1 (2).

The close relationship between Aflatoxin intake and human liver cancer has been demonstrated in various areas and the regulatory level for Aflatoxin B_1 in foods adopted in many countries is 5 ppb. The accepted limits of AFB_1 and total Aflatoxins in foods are 5 and 10 µg/kg, respectively in more than 75 countries around the world whilst, they are 2 and 4 µg/kg in the European Union (3,4).

The objective of this study was to determine the residual content of AFB_1 in breast and thigh meat samples of imported and local broiler carcasses present in Kurdistan market.

Materials and methods

Breast and thigh broiler muscle samples were collected from Kurdistan meat markets, farms and poultry slaughterhouses through the period from 3 March 2013 to 26 November 2013. They were 10 breast and 10 thigh muscle samples of Brazilian broiler carcasses (hilal and Sadia); 5 breast and 4 thigh muscle samples of Iranian broiler carcasses (Khosh pokht); 30 breast and 30 thigh muscle samples of Turkish broiler carcasses (Yam-tapilic, Lades, Senplic, Kapidac, Kozoa and Oznesilpilic); and 45 breast and 45 thigh muscle samples of locally reared broiler carcasses. Samples were frozen at -20 °C for subsequent ELISA analysis of residual AFB_1.

ELISA kit

ELISA kit for determination of residual AFB_1 in breast and thigh meat samples was used (Shenzhen Lvshlyuan Biotechnology Co., Ltd Green spring, versio: 2012-02). This test kit was based on the competitive enzyme immunoassay for the detection of AFB_1. The coupling antigene is pre-coated on the micro-well strips. The AFB_1 in the sample and the coupling antigens pre-coated on the micro-well strips compete for the anti-AFB1 antibodies. After the addition of the enzyme conjugate, the TMb substrate is added for coloration. The optical density (OD) value (three times reading with Epson LQ-300$^+$ II) of the sample has a negative correlation with the AFB_1 in the sample. This value is compared to the standard curve and the AFB_1 residue is subsequently obtained.

Sample preparation

Five grams of each sample were weighed and homogenized. To the grounded sample 20 ml of hexan were added, followed by 25 ml of methanol solution (1 part of methanol+ 1 part of water). The mixture was shacked violently for 15 min and left to be settled for 5 min. The clear liquid layer part was transferred into separatory funnel. After that the liquid was shacked for 2 min and left for stratification. The lower methanol layer was taken directly for analysis.

Procedure

Operation procedure was done according to the instruction given by the manufacture (Shenzhen Lvshlyuan Biotechnology Co., Ltd). Quantitative analysis was obtained by software and AFB1 was calculated according to the formula

AFB1(ng/g)= C x V/ m x D

C: AFB1 content (ng/ml) of the sample, V: Sample extract solution volume (ml), M: Sample quantity (g), D: Sample dilution factor.

Results

AFB_1 residues

The overall picture of AFB_1 residues in imported broiler breast and thigh positive samples are presented in figures 1. From the figure, it is evident that out of 89 imported samples, 21 (23.59%) were detectable or positive (containing 0.5 ppb AFB_1 and more), while the remaining 68 samples (76.40%) were undetectable or negative (containing less than 0.5 ppb AFB_1) to ELIZA test. The overall picture of AFB_1 residues in imported broiler breast and thigh acceptable (containing <5 ppb AFB_1) and rejected samples (containing > 5 ppb AFB_1) are presented in figures 2. From figure 2, it is evident that out of 89 imported samples, only 2 (2.24%) were rejected, while the remaining 87 samples (97.75%) were acceptable**.**

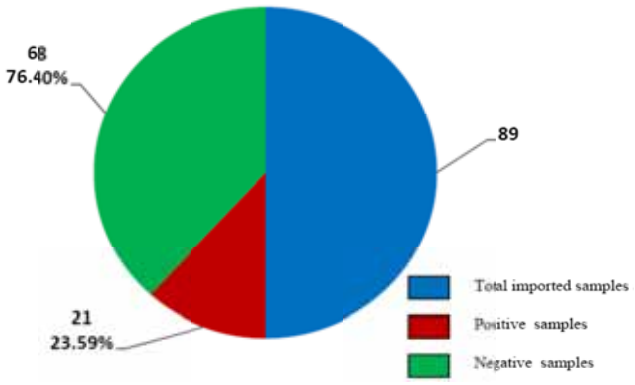

Figure 1: Percentage of positive and negative imported broiler breast and thigh muscle samples for residual AFB_1.

The overall picture of positive residual AFB_1 in local broiler breast and thigh samples are presented in figures 3. As the figure shows, it is evident that out of 90 local samples, 19 (21.11%) were detectable or positive (containing 0.5 ppb AFB_1 and more), while the remaining

71 samples (78.88%) were undetectable or negative (containing less than 0.5 ppb AFB_1) to ELIZA test.

The overall picture of AFB_1 residues in local broiler breast and thigh acceptable (containing <5 ppb AFB_1) and rejected samples (containing > 5 ppb AFB_1) are presented in figures 4. As the figure shows, it is evident that out of 90 imported samples, 10 (11.11%) were rejected, while the remaining 80 samples (88.88%) were acceptable**.**

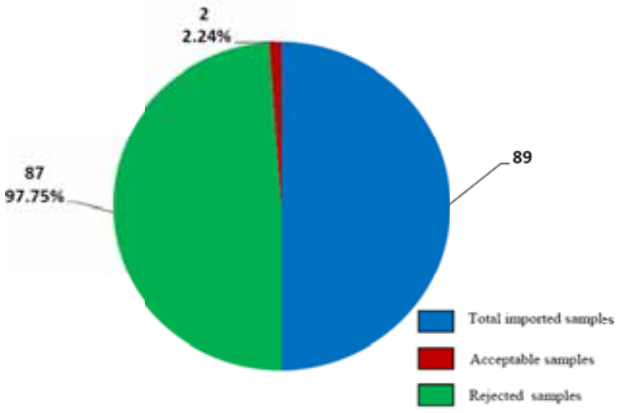

Figure 2: Percentage of acceptable and rejected imported broiler breast and thigh muscle samples for residual AFB_1.

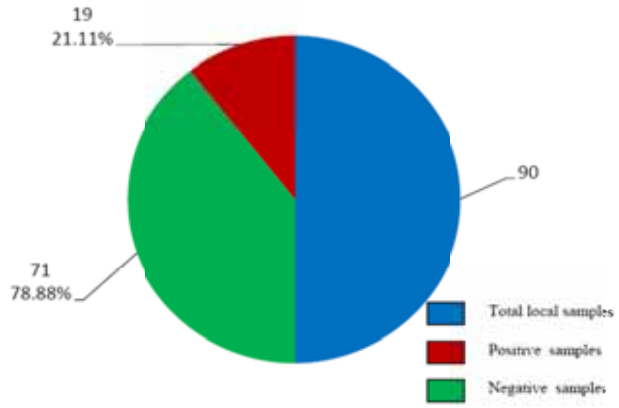

Figure 3: Percentage of positive and negative local broiler breast and thigh muscle samples for residual AFB_1.

The results for residual AFB_1 in broiler breast and thigh samples imported from Iran are shown in Table 1. Out of the total 9 samples, no positive breast and thigh muscle samples were detectable or positive (containing 0.5 ppb AFB_1 and more), and all the remaining 9 samples (100%) were negative (containing less than 0.5 ppb AFB_1). All breast and thigh samples contained less than 5 ppb of USA regulations about the permissible level of AFB_1 in foods intended for human consumption. The results of residual AFB1 in broiler breast and thigh muscle samples imported from Turkey are shown in Table 1. Out of the total 60 samples, 12 (20%) were detectable (positive, containing 0.5 ppb AFB_1 and more), while the remaining 4 samples (80%) were undetectable (negative, containing less than 0.5 ppb AFB_1). For detailed results, out of 30 breast meat samples tested, 8 (26.66%) were detectable (positive, containing 0.5 ppb AFB_1 and more), while the remaining 22 samples (73.33%) were undetectable (negative, containing less than 0.5 ppb AFB_1). The results with the thigh muscle samples showed that 4 samples (13.33%) were detectable (positive, containing 0.5 ppb AFB_1 and more) out of the 30 samples tested, while 26 samples were undetected (86.66%) (negative, containing less than 0.5 ppb AFB_1). Only one breast sample has residual value more than 5 ppb of the USA regulations about the permissible level of AFB_1 in foods intended for human consumption.

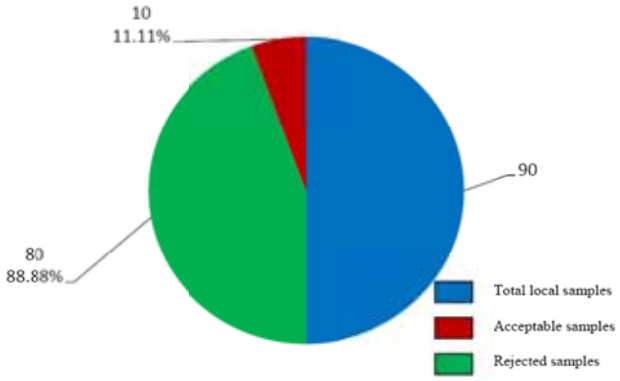

Figure 4: Percentage of acceptable and rejected local broiler breast and thigh muscle samples for residual AFB_1.

The results for residual AFB_1 in broiler breast and thigh samples imported from Brazil are shown in Table 1. Out of the total 20 imported samples, 9 samples (45%) were detectable (positive, containing 0.5 ppb AFB_1 and more), while the remaining 11 samples (55%) were undetectable (negative, containing less than 0.5 ppb AFB_1). For detailed results, out of 10 breast muscle samples tested, 5 (50%) were detectable (positive, containing 0.5 ppb AFB_1 and more) while the remaining 5 samples (50%) were undetectable (negative, containing less than 0.5 ppb AFB_1). Out of 10 thigh muscle samples tested, 4 (40%) were detectable (positive, containing 0.5 ppb AFB_1 and more) while the remaining 6 samples (60%) were undetectable (negative, containing less than 0.5 ppb AFB_1). Nineteen breast and thigh muscle samples contained less than the permissible level of residual AFB_1 for human consumption of 5 ppb AFB_1 (USA, regulation). Only one breast sample has residual AFB1 value more than the permissible level of residual AFB_1 for human consumption of 5 ppb AFB_1 (USA, regulation).

Table 1: Percentage of positive accepted and rejected breast and thigh muscle imported samples

Producer	Type of muscle	No. of samples tested	No. positive 0.5 ppb AFB_1 and more	%	Range ppb	Accepted < 5ppb	Rejected >5ppb
Iran, Khosh pokht	Breast	5	0	0	0	5	0
	Thigh	4	0	0	0	4	0
	Total	9				9	0
Turkey, Yam-tapilic	Breast	5	0	0	0-0.1	5	0
	Thigh	5	0	0	0-0.1	5	0
	Total	10				10	0
Turkey, Lades	Breast	5	2	40	0-1.334	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Turkey, senpilic	Breast	5	1	20	0-0.515	5	0
	Thigh	5	1	20	0-0.515	5	0
	Total	10				10	0
Turkey, Kapidac	Breast	5	5	80	0-6.698	4	1
	Thigh	5	3	60	0-3.22	5	0
	Total	10				9	1
Turkey, Kozoa	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Turkey, Oznesilpilic	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Brazil, hilal	Breast	5	5	100	1.546-6.080	4	1
	Thigh	5	4	80	0.645-2.974	5	0
	Total	10				9	1
Brazil, Sadia	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0

The results for residual AFB_1 in local broiler breast and thigh muscle samples are shown in Table 2. Out of the total 90 samples, 19 (21.11%) were detectable (positive, containing 0.5 ppb AFB_1 and more), while the remaining 71 samples (78.88%) were undetectable (negative, containing less than 0.5 ppb AFB_1). For detailed results, out of 45 breast muscle samples tested, 9 samples (20.00%) were detectable (positive, containing 0.5 ppb AFB_1 and more) while the remaining 36 samples (80.00%) were undetectable (negative, containing less than 0.5 ppb AFB1). Out of 45 thigh muscle samples tested, 10 samples (22.22%) were detectable (positive, containing 0.5 ppb AFB_1 and more) while the remaining 35 samples (77.77%) were undetectable (negative, containing less than 0.5 ppb AFB_1). Eighty (88.88%) of breast and thigh muscle samples contained less than 5 ppb according to USA regulations regarding the permissible level of AFB_1 of foods intended for human consumption, while 10 (11.11%) were rejected, since they contained more than 5 ppb of the USA regulations.

Discussion

The detectable (positive, containing 0.5 ppb AFB_1 and more) percentages of residual AFB_1 in breast muscle samples were almost similar to those of the thigh muscle samples in the imported (Iran, Turkey and Brazil) and also in that local samples. The presence of detectable samples indicated that these birds from which tested samples were taken were subjected to AFs contamination in their diets through their rearing period. The overall percentages of detectable residual AFB_1 in imported and local breast and thigh samples were lower than those of undetectable (21 vs. 68%) and of (19% vs.71%) respectively. However, more rejectable samples were recorded in local breast and thigh samples (10 (11.11%)), compared to the imported samples (2 (2.24%)). These results indicated to the high health risk concern due to residual AFB_1 contamination in local samples compared to that of imported ones. It should be stressed here that most of the original AFB_1 consumed by the birds has already been converted in their livers to many other metabolites especially AFM_1 (2) which need another

work concentrated on the estimation of residual AFM_1 in breast and thigh muscle in addition to its presence in liver, since the residual level of AFM_1 in muscles and liver is always higher than that of AFB_1 (3).

From the above tested samples, especially of the local ones, they contained more than 5 ppb residual AFB_1 and some reached 15.2456 ppb AFB_1, making them unfit for human consumption. (4,5).

Addition of detoxifying agents or adsorbents (5) to poultry feeds are of urgent demands as an effective means for reducing residual Aflatoxins and other mycotoxins in poultry carcasses and subsequently in reduction of the consumers health problems through the food chain.

Table 2: Percentage of positive accepted and rejected breast and thigh muscle local samples

Producer	Type of muscle	No. of samples tested	No. positive 0.5 ppb AFB_1 and more	%	Range ppb	Accepted < 5ppb	Rejected >5ppb
Hoshiar poultry farm	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Nihad poultry farm	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Hokar poultry farm	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
AL-Eetimad poultry slaughterhouse	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Mansoor poultry farm	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
AL-Shimal poultry house No.2	Breast	5	4	80	0-15.2456	2	3
	Thigh	5	5	100	1.216-10.246	3	2
	Total	10				5	5
AL-Shimal poultry house H	Breast	5	5	100	1.568-6.025	4	1
	Thigh	5	5	100	3.866-6.628	1	4
	Total	10				5	5
Mardin poultry house G	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0
Mardin poultry house I	Breast	5	0	0	0	5	0
	Thigh	5	0	0	0	5	0
	Total	10				10	0

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