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RESEARCH ARTICLE

EVALUATION THE DIFFERENT METHODS DETECTION OF AFB_1 IN ANIMALS FEED IN IRAQ

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ABSTRACT

The 145 samples were collected from different region from Iraq during March 2014 – February 2015, distributed into 55 samples imported and 90 samples local markets include: corn, wheat, malt, soybeans, compete cow's feed and feed additives. The samples were analysis to quality and quantity detection of aflatoxin B_1 (AFB_1) by using methods of Thin layer chromatography(TLC), High performance liquid chromatography (HPLC) and Enzyme Linked Immune Sorbent Assay (ELISA) techniques. The results from quality detection of AFB_1 by TLC showed the 30% of samples were positive, While the quantity and quality detection of HPLC and -ELISA techniques results appeared 44.8 % and 41 % of samples respectively were positive (contaminated with AFB_1), furthermore the results showed the corn, complete cow's feed and feed additives samples more contaminated samples with AFB_1. The highest concentration of AFB_1 found in imported compete cow's feed at 100% were positive with AFB_1 ranged from 70.5-86.43 ng/ g in HPLC and 76.1-95.1 ng/ g in ELISA whereas in local samples was found feed additives with high concentration of AFB_1 at 75% in HPLC and 50% in ELISA were ranged from 1.1 to 817.9 ng/g and 3.32 to 111.47 ng/g in HPLC and ELISA technique respectively while the soy sample was lesser contaminate. The contamination rate was high in the local samples compared to imported samples. Also the results observed that no significant differences between the HPLC and ELISA techniques, but have significant differences when compared with TLC technique, that may be refer into the sensitivity and specificity of HPLC and ELISA were higher than the TLC.

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INTRODUCTION

Aflatoxins are a group of structurally related toxic compounds produced by the fungi species *Aspergillus* spp., Principally *Aspergillus flavus* and *A. parasiticus*, rarely by *Aspergillus nomius* Wild and Gong 2010; Iqbal, *et al*., 2012; Iqbal and Asi, 2013). Aflatoxin may be infect major food groups such as: corn, rice, wheat, and nuts that lead to the poor quality of agricultural products, thus causing great economic loss to the countries. (Liu and Wu, 2010). In addition they are infected animal farming during consumption feed contamination with AFB_1 lead to reduced performance and increased susceptibility to infections (Bondy and pestka, 2000). Aflatoxin B_1 (AFB_1) was shown to have the most toxic and carcinogenic properties to humans and animals and it has been involved with the development of human hepatic and extra hepatic carcinogenesis (Iqbal *et al*., 2012, (Hammami *et al*., 2014)).

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The European Committee Regulations (ECR) has established the maximum level of AFB_1 in cereal, peanut, and dried fruits for direct human consumption in 4 ng/g for total aflatoxin and 2 ng/ g for AFB_1 alone and 15-20 ppb in animals feed products according to Food and Drug Administration (FDA) and U.S.A. Department of Agriculture (Alcaide -Molina *et al*., 2009), Where there is no acceptable daily Intake (ADI) for aflatoxin, because it is a genotoxic and carcinogenic substances, exposure through food must be kept at the lowest possible level (EFSA, 2013). The aim of the study using different methods to determination of AFB_1 such as Thin Layer Chromatography (TLC) as quality detection while using High performance liquid chromatography (HPLC) and Enzyme Linked Immune Sorbent Assay (ELISA) (Almeida *et al*., 2011) as quality and quantity determinant, because of lesser objective studies in Iraqi. Therefore we research into evaluate the presence of AFB_1 in local and imported agriculture feed samples by using the TLC, HPLC and ELISA techniques to compared between them methods for determination the concentration of AFB1 in samples.

MATERIALS AND METHODS

Samples Collection

A total 145 imported and local dairy animal's feed samples were randomly collected from localized feed markets in different region of Baghdad during March 2014 to February 2015. The samples included: Corn , Wheat, Malt , Soybeans , Compete cow's feed and Feed additives as No. (15, 5, 10, 10, 5 and 10) imported samples respectively, and No. (20, 15, 10, 10, 15 and 20) local samples respectively. Two kilogram of each sample was placed in plastic bags to be avoid any moisture, then transferred to the laboratory, and ground in a laboratory mill to pass a 1.0 mm, screened then mixed accurately to ensure homogeneity. If not analyzed immediately, samples were kept in plastic bags and stored at -20°C (AOAC, 2005 ; Xiang *et al.,* 2006).

Aflatoxin B_1 Standard Curve

The standard AFB_1 solution was prepared according to AOAC 2000a) with some modification in acetonitrile at a concentration of 25 µg/ml to prepare stock solution and kept at -20 ^{0}C. The standard curve drawn with concentrations (10, 5, 2 and 1.25) µg/ ml of AFB_1 apposite area by using HPLC technique Fig.1.

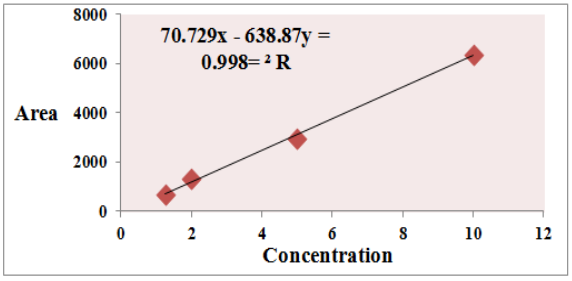

Fig.1: Standard curve of AFB_1 concentrations by using HPLC technique

Extraction of Aflatoxin B_1

The feed and grains where extracted according to (AOAC, 2000b) with some modification, when used methanol (90%) and hexane (1:1 v/v) in separation method.

Quantity detection of AFB1.

The quantity analysis of AFB1 were detect by using HPLC according AOAC (1990). The concentration of aflatoxin for each sample could be measured by application area of any peak from HPLC analysis in the standard curve equalities to gain the AFB1 concentration of the samples. For determination AFB_1 by ELISA The reagent and samples must be prepared according to the recommended Bioscientific Kit instruction. The samples 5 g were ground, add 25 ml of 70% methanol and shaker and centrifuges for 10 minutes at 4000 rpm. Dilute 1 ml of the obtained supernatant with 1 ml of 1x PBS. Vortex the samples well then use 50 µl of the diluted supernatant per well in the test. The determination of AFB_1 can be calculated by using special program with Excel functionality for Bioscientific Company.

Statistical Analysis

The statistical analysis was conducted to extract the Mean ± Standard Error. The averages were tested using polynomial Duncan test (Duncan, 1955). Test the differences between the averages in the experiences of the effectiveness of different Numbers separately compared to the control using T-test (17) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Quality and quantity detection of AFB1

Table (1) shown number and (percent) of positive samples when detected by TLC technique at 55 samples (37.9%) while the negative samples 90 (62.1%) respectively from 145 samples collected. Also the result in the same table appear the compete cow's feed (75%), corn (57.1%) and feed additives (33.3%) of imported and local samples have more contamination samples, while the soybean samples cannot be AFB_1 detected by this technique Fig. 2.

Table 1: Results of TLC Technique of AFB_1 in different samples of feed and its contents

Sample Category	Source of Sample	No. Sample	Positive Sample		Negative sample	
			No.	(%)	No	(%)
Corn	Imported	15	10	66.7	5	**33.3**
	Local	20	10	50	10	**50**
Wheat	Imported	5	0	0	5	**100**
	Local	15	5	33.3	10	**66.7**
Malt	Imported	10	5	50	5	**50**
	Local	10	0	0	10	**100**
Soybean	Imported	10	0	0	10	**100**
	Local	10	0	0	10	**100**
Compete cow's feed	Imported	5	5	100	0	**0**
	Local	15	10	66.7	5	**33.3**
Feed additives	Imported	10	0	0	10	**100**
	Local	20	10	50	10	**50**

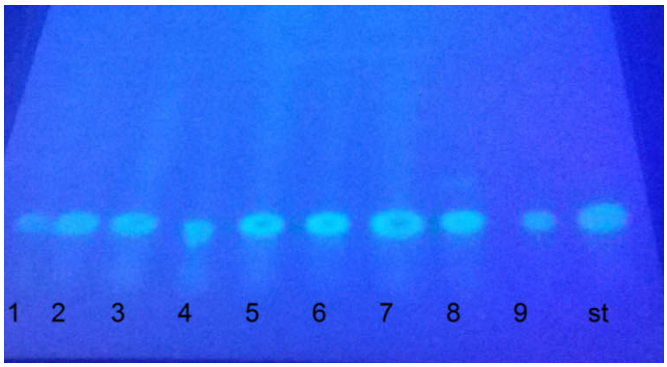

Fig. 2. In all samples are contaminated with AFB1 when compared with standard, the spots 1-3 of imported samples (corn, malt and compete cow's feed), while spots 4-9 of local samples (corn, wheat, Compete cow's feed and feed additives).

Table (2) The HPLC results showed the concentration AFB_1 in the different samples were ranging from 0.57 – 817.9 ng / g, the concentration of samples when reached up to 15 ppb refer to positive results according to U.S. Department and Agriculture and U.S. Food and Drug Administration (Ricci *et al.,* 2007). The imported samples of compete cow's feed (100 %), malt (50%) and corn (33.3%) were contaminated with AFB_1 more than local samples feed additives (75%), compete cow's feed (66.7%) and wheat and (50%) in corn and malt. The AFB1 concentration in local feed additives, wheat and corn ranged from 1.1 to 817.9, 1.14 to 403 and 2 to 297.8 ng/g while the mean values were 408.9, 135 and 100.97 ng/ g respectively. Whereas the concentration of AFB1 in imported compete cow's feed, malt and corn were ranged from 70.5 to 86.43, 6.7 to 92

and 1.2 to 15.3 ng/ g were the mean values reached at 78.5, 35.2 and 7.2 ng/ g respectively. The Table (3), showed the ELISA technique results of AFB_1 concentration rang reached 0.001 to 111.47 ng/ g in local and imported samples. The imported samples (compete cow's feed 100%, corn 66.7% and soybean and malt 50%), while in local samples (corn, malt, feed additives 50%, wheat and Compete cow's feed 33.3%). The local samples feed additives, corn, wheat and compete cow's feed have high concentration of AFB_1 were ranged from 3.32 to 111.47, 10 to 79.01, 0.001 to 64.17 and 2.3 to 54.21 ng/ g, while the mean values were 55.32, 34.58, 21.79 and 21.17 ng/g respectively. The ranged and mean values for compete cow's feed, corn, and malt were reached at 75.1 to 95.1, 9.72 to 58.56 and 12.3 to 34.73 ng/g and 85.1, 30.33 and 26.5 ng/g respectively. From fig. 3 and 4, results of HPLC technique appear the highest positive percentage were 44.8%, While in ELISA technique were 41.3% and in TLC was only ones reached 37.9%. The ELISA has highest performance than HPLC technique with 0.001 ng/ g in local wheat samples, but HPLC technique can detect the highest concentration of AFB_1 in local feed additives with 817.9 ng/ g. From the results of three techniques, the samples of compete cow's feed and corn were considered higher contaminated samples while soybean was less contaminated.

The high level of aflatoxin in compete cow's feed, feed additives and corn were causes when found moisture, badly storage conditions, and poor management, that affect on the rate of aflatoxin producer (Kabar *et al*., 2006). Kitya *et al*.(2010) observed that the level of aflatoxin in the food samples (groundnuts, cassava, millet, sorghum flour) In Uganda ranged from 0 to 55µg/kg. In addition, the study of Hassan *et al*.(2014) were detected AFB_1 in 12 out of 24 maize samples when collected from Iraq government ranged from 2.30 to 30 ppb in TLC technique and 270 to 500 ppb in ELISA technique. Some countries such as Tunisia and Lebanon have been reported high levels of contamination of wheat and their derivatives with aflatoxin (Aydin *et al*., 2008; Joubrane *et al*., 2011 and Almeida *et al*., 2013). As for the three technologies used in the detection and determination of aflatoxin (Pirestani *et al*. (2011) was concluded that there was no significant difference between the values obtained by the Elisa and HPLC. However sensitivity and specificity of HPLC was higher than the Elisa method. In addition Huang *et al*. (2010) concluded that TLC technique was poor separation, unsatisfied accuracy and low sensitivity limit while ELISA method is performed in determination aflatoxin for being fast and sensitive method for aflatoxin analysis.

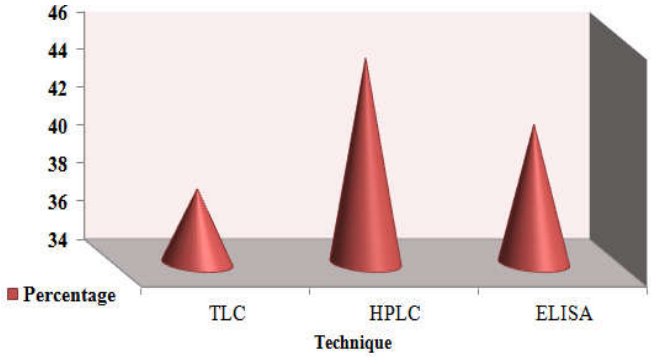

Fig. 3: Comparison results of TLC, HPLC and ELISA Techniques in determinate of AFB1

Table 2: Results of HPLC Technique of AFB_1 in different samples of Feed and its contents

Sample Category	Source of Sample	No. Sample	Positive Sample		Range	(Mean ± SE)
			No.	(%)	Min. – Max.	
Corn	Imported	15	5	33.3	1.2 – 15.3	7.2 ± 3.6
	Local	20	10	50	2 – 297.8	100.97 ± 85.2
Wheat	Imported	5	0	0	0.57 – 0.63	0.6 ± 0.015
	Local	15	10	66.7	1.14 – 403	135.4 ± 116
Malt	Imported	10	5	50	6.7 – 92	35.2 ± 24.5
	Local	10	5	50	3.9 – 5	4.6 ± 0.3
Soybeans	Imported	10	0	0	2.9 – 8.3	5.8 ± 1.4
	Local	10	0	0	1.2 – 9.8	2.3 ± 0.7
Compete cow's feed	Imported	5	5	100	70.5 – 86.43	78.5 ± 3.9
	Local	15	10	66.7	2.4 – 41.2	15.41 ± 11.1
Feed additives	Imported	10	0	0	1.7 – 5.21	4 ± 1
	Local	20	15	75	1.1 – 817.9	408.9 ± 204

Table 3: Results of Elisa Technique of AFB_1 in different samples of Feed and its contents

Sample Category	Source of Sample	No. Sample	Positive Sample		Range	(Mean ± SE)
			No	(%)	Min. – Max.	
Corn	Imported	15	10	66.7	9.72 – 58.56	30.33 ± 12.6
	Local	20	10	50	10 – 79.01	34.58 ± 19.3
Wheat	Imported	5	0	0	2.48 – 3.4	3 ± 0.24
	Local	15	5	33.3	0.001 – 64.17	21.79 ± 18.4
Malt	Imported	10	5	50	12.3 – 34.73	26.5 ± 6.2
	Local	10	5	50	2.3 – 10.94	7.74 ± 2.4
Soybeans	Imported	10	5	50	0.02 – 23.14	14.9 ± 6.5
	Local	10	0	0	1.99 – 3.01	2.5 ± 0.3
Compete cow's feed	Imported	5	5	100	75.1 – 95.1	85.1 ± 5
	Local	15	5	33.3	2.3 – 54.21	21.17 ± 14.5
Feed additives	Imported	10	0	0	2.16 – 14.81	9.9 ± 3.4
	Local	20	10	50	3.32 – 111.47	55.32 ± 27.1

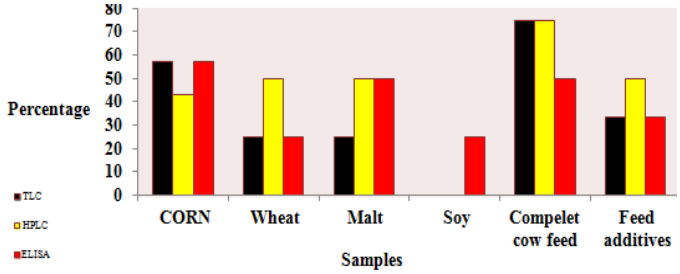

Fig. 4: The compression percentage results of contamination samples with AFB1 by using TLC, HPLC and ELISA

Conclusion

Uses the TLC, HPLC and ELISA techniques to AFB1 detection in local and imported samples of compete cow's feed and its content are proved the efficiency of those technologies, while showed differences in efficiency between them in the detection activity, It can reason lies in the difference in the technical quality and the mechanism of action. In addition detect most of samples contain AFB1 more than 15 ng/ kg exceeding the acceptable limit in many countries and the local samples has been more contaminated with AFB1.

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