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DETECTION OF AFLATOXIN IN COMPOUND FEEDS OF BROILER FLOCKS SUFFERED FROM FIELD AFLATOXICOSIS

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ABSTRACT

One hundred and fifty eight broiler compound feed samples were delivered from broiler flocks in Ninevah governorates. These flocks were clamed to be affected with field aflatoxicosis. All the examined samples had aflatoxin levels higher than of the permissible limit (20 ppb). The range of AF levels was 22-2263 ppb. The mean value of AF concentration was 592.7ppb. The percentage of AF concentrations from 0-500 ppb was 48.7%, and from 500-1000 ppb was 41.2%, while that from 1000-2500 was 10.2%.

الكشف عن سموم الافلا في اعلاف فروج اللحم للقطعان المصابه بالتسمم بسموم الافلا الحقلي

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الخلاصة

تم فحص 185 عينه اعلاف جلبت من اصحاب حقول فروج اللحم في محافظه نينوى اشتكي اصحابها من احتمال التسمم بسموم الافلا . احتوت جميع العينات المفحوصه على سموم الافلا بمستوى اعلى من الحد المسموح به وهو20 جزء بالبليون . وكان مستوى سموم الافلا يتراوح بين22–2263 جزء بالبليون وبمعدل 592.7 جزء بالبليون. كانت نسبه مستويات التلوث بين0–500 جزء بالبليون هي48.7 % وبين 500–1000 هي41.2 % بينما كان للمستويات بين 1000–2500 هي10.2%.

INTRODUCTION

Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by certain strains of *Aspergillus flavus* and *A. parasiticus*; in particular , *Aspergillus flavus* is a common contaminant in agricultural commodities. *Aspergillus bombycis*, *Aspergillus ochraceoroseus* , *Aspergillus nomius*, and *Aspergillusp seudotamari* are also aflatoxin-producing species, but they are encountered less frequently (1). Aflatoxins are a family of extremely toxic,

mutagenic, and carcinogenic compounds (2). Toxigenic A.flavus isolates produce aflatoxins B1, and B2 and toxigenic A. parasiticus isolates produce aflatoxins B1, B2, G1, and G2 (3). Many substrates support growth and aflatoxin production by aflatoxigenic molds. Natural contamination of cereals oilseeds, nuts, and a long list of other commodities is a continuing worldwide problem (4). Crops could be contaminated with aflatoxin in the field before harvest, where it is usually associated with drought stress (5); even more problematic is the fate of crops stored under conditions that favor mold growth. In storage, usually the most important variables are the moisture content of the substrate and the relative humidity of the surroundings (6). *Aspergillus flavus* is the predominant fungus in aflatoxin – contaminated corn, and with *A. parasiticus* are temperature-tolerant fungi and can be selectively isolated on a high salt culture medium incubated at 37 C^0(7). Aflatoxin contamination has been linked to lowering resistance to diseases and interfering with vaccine-induced immunity and increased mortality in poultry, and also significantly lowers the value of grains as animal feed, (8). Few surveys on the occurrence of aflatoxins in poultry feeds have been conducted. Jindal *et al.,* (1993) (9) analyzed 240 poultry feeds from India. All samples were positive for aflatoxins with levels ranging from 7 to 11,600 µg/kg (ppb). Levels higher than 30 ppb were detected in 76% of the samples. On the other hand, aflatoxin levels of 30-1610 ppb were found in 19% of 31 samples of compound poultry feed in Nigeria (10), while 91% of 34 samples of poultry feed in Indonesia contained aflatoxin levels ranging from 22 to 6171 ppb (11). Hegazy *et al.,* (1991) (12) reported that 30.7% of 1175 poultry feed samples collected from Egyptian farms were contaminated with aflatoxin . The concentration of aflatoxin in the positive samples ranged from 1 to 2000 ppb. In Mosul province(Iraq), it was found that out of 450 broiler mixed feed samples, 66% were positive to one or more of aflatoxins B1,B2,G1, and G2 during four years of study 1999-2003(13). The present study was aimed to estimate AF levels in compound feeds of broiler flocks clamed to be suffering from natural outbreaks of field aflatoxicosis in the northern governorates of Iraq.

MATERIALS AND METHODS

Feed sampling: One hundred and fifty eight samples of ground broiler compound feed samples in approximately 1kg were delivered from different broiler farms located in Ninevah governorate, showing signs and post-mortem changes of aflatoxicosis, during the period 2003-2005.

Aflatoxin assay:

The levels of aflatoxin contamination of feed samples were determined by the method of direct competitative enzyme-linked immunoassay using Neogen extraction kit (Neogen Corporation) as follows:

1-Sample preparation and extraction:

Twenty five gram- samples were collected for analysis. These samples were finely ground, so that at least 75% of them pass through a 20 mesh. After grinding, 5 gram samples were blended with 25 ml of 70% v/v methanol/water solution (7 parts methanol/3 parts deionized water) for 3 minutes. Extracts were filtered through a Whattman no.1 filter paper. The filtrates were then collected.

2-Test procedure:

All Neogen extraction reagents were allowed to warm at room temperature (18-30C) before use. Red marked mixing wells were prepared, one for each

sample plus four red wells for controls 0,5,15 and 50 ppb. All red-marked wells prepared were placed in the well holder. An equal number of antibody-coated (AB) white wells to those red –marked wells were also prepared. Hundred µl of conjugate were transferred to each red-marked mixing well. To those red wells containing the conjugate another (with new pipette tips) 100µl of controls and samples were added by using a 12-cannel pipettor liquid in wells were mixed by pipetting it up and down for 3 minutes. After mixing 100µl of the (conjugate+ samples, or conjugate + controls) were transferred to AB –coated wells. These wells were moved back and froth for well mixing the contents in each well for 10-20 seconds without splashing reagents from the wells. Antibody-coated wells were then incubated at room temperature (18-30 C^0). The contents in AB-coated wells were shacked out, by filling the wells with deionized water and dumping them out. This step was repeated 5 times. Turning the wells upside down and tamping them out on a paper was carried out until the remaining water has been removed. Substrate was then added to AB –coated wells, by using the 12-channel pipettor through pipetting 100µl of substrate to these wells. Mixing was done by sliding the well holder back and froth for 10-20 seconds, followed by incubation for 3 minutes. Stop solution was poured to these wells (100µl) to each, mixing was done by sliding well holder back and forth on a flat surface. Within 20 minutes after the addition of stop solution. Results were read, using a micro well reader (Elx800) with a 650 nm filter. More blue color means less aflatoxin . Results of the yield optical densities of the controls and samples were obtained by using computerized Neogen Verotex Software program version 2.0.16 (Neogen Corporation).

Statistical analysis: The data were analyzed using computerized statistical program (SPSS, 2005 (14).

RESULTS

Post-mortem findings:

Necropsy findings of some broilers delivered with mixed feeds they consumed, show enlarged pale liver and kidneys(Figure 1,2 and 3). Discolored livers were ranged from clay to yellow color, owing to fat accumulation in hepatocytes. Livers also show sub capsular hemorrhages, focal areas of necrosis, and many of them were friable(Figure 4). Gall bladders were full, and the intestines were filled with catarrhal contents. Many necropsed birds were also exhibited varying degrees of skeletal myopathy .

Aflatoxin levels:

The entire broiler compound feed samples, which delivered from farmers clamed from mycotoxicosis in their broiler flocks, attempting to clarify the presence of mycotoxins in their delivered feed samples for detection of mycotoxins (here aflatoxin) show that there was surprisingly high levels of aflatoxin contamination (Table 1).

Levels were ranged from 22 ppb to 2263 ppb, with a mean value of 592.7 ppb, with a median value of 522(Table 2). Most of the obtained AF concentrations were scattered between 22 to 1000 ppb

It could be collectively said that higher number of AF contaminated samples(77 samples) had AF values from 0-500 ppb, and 65 samples had AF levels of 500-1000 ppb, while only 16 samples had the remaining AF levels of 1000-1500 ppb

(6 samples), 1500-2000ppb (5 samples), and 2000-2500 ppb (5 samples) respectively.

To explore these numbers in percentages, it is evident from Figure 5, that the highest percentage (17%) was obtained in samples with AF concentration ranged from 100-200 ppb. From 3-10% were all the samples with each AF concentrations between 0-1000ppb (except those of levels from 100-200 ppb). Two and lower percentages were experienced in the remaining AF levels (1000-2500 ppb).

Figures; 1, 2, 3 and 4, shows Lethal aflatoxicosis in broiler chicken causing liver and kidney discoloration, from clay to yellow liver, owing to fat accumulation in hepatocytes, with sub capsular hemorrhage and focal areas of necrosis. Aflatoxin (2218 ppb) was detected in the mixed feed offered to these broilers.

Figure 1: Enlarged pale liver of broiler chick with aflatoxicosis

Figure 2: Enlarged pale liver and kidneys of broiler chick with aflatoxicosis

Figure 3: Enlarged pale liver and kidneys of broiler chick with aflatoxicosis

Figure 4: Livers also show sub capsular hemorrhages, focal areas of necrosis of broiler chick with aflatoxicosis.

The cumulative percentage of AF contaminated samples with levels between 0-500 ppb was 48.7%, and those with levels ranged from 500-1000 was 41.2, while only 10% were the cumulative percentages of the remaining AF levels 1000-1500ppb (3.8%), 1500-2000ppb (3.1%), and 2000-2500ppb (3.2%) respectively (Figure 6).

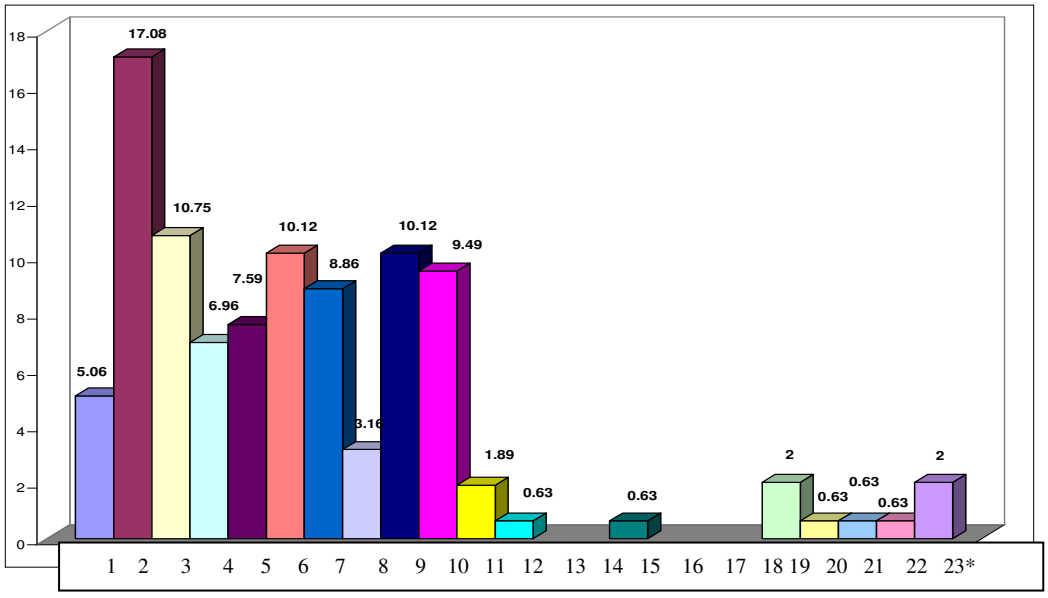
The entire tested broiler mixed feed samples had AF levels higher than the permissible limit for broilers of 20 ppb.

Table 1: Total broiler mixed feed samples, delivered from different broiler flocks, suffering from aflatoxicosis, in all governorates involved, and the concentrations of aflatoxin (ppb) in these samples.

Sample	AF PPb	Sample	AF PPb	Sample	AF PPb	Sample	AF PPb	Sample	AF PPb	Sample	AF PPb
1	22	28&29	173	57	313.0	85	563.00	112	802	138	965
2	59	30	175	58	326.00	86	566.00	113	812	139	980
3	81	31	180	59&60	342.00	87	575.00	114	820	140	990
4	84	32	182	61	345.00	88&89	580.00	115	831	141	996
5	86	33&34	184	62	372.00	90	581.00	116	838	142	998
6	89	35	195	63	378.00	91	586.00	117& 118	845	143	1004
7&8	90	26&37	196	64	389	92	593	119	847	144	1010
9	100	38	201	65	390	93	598	120	858	145	1020
10	102	39	203	66	418	94	619	121	859	146	1022
11	103	40	209	67	423	95	627	122	862	147	1128
12	104	41	212	68	436	96	631	123	873	148	1458
13	109	42	221	69	440	97	648	124	879	149	1668
14	112	43	222	70&71	445	98	652	125	889	150	1731
15&16	113	44&45	226	72	450	99	657	126	891	151	1827
17	117	46	231	73&74	468	100& 101	659	127	895	152	1845
18	119	47	232	75	481	102	667	128	900	153	1957
19&20	126	48	235	76	486	103	681	129	906	154& 155	2081
21	146	49	252	77	495	104	692	130	922.00	156	2113
22	150	50&51	259	78	504	105& 106	695	131	923.00	157	2218
23	152	52	280	79	518	107	706	132	928.00	158	2263
24	154	53	286	80	526	108	721	133& 134	940.00		
25	156	54	289	81	527	109	731	135	945.00		
26	158	55	304	82	534	110	732	136	950.00		
27	168.0	56	310	83&84	540	111	768	137	963.00		

Table 2: Maximum, minimum, and median aflatoxin concentrations (ppb), in all tested compound feed samples.

			Statistic	Std. Error
VAR00001	Mean		592.6899	37.85440
	95% Confidence Interval for Mean	Lower Bound	517.9203	
		Upper Bound	667.4595	
	5% Trimmed Mean		540.4409	
	Median		522.0000	
	Variance		226406.967	
	Std. Deviation		475.82241	
	Minimum		22.00	
	Maximum		2263.00	
	Range		2241.00	
	Interquartile Range		642.2500	



* Figure 5: Percentages of AF contaminated samples distributed according to their AF levels

1= 0-100 2=101-200 3=202-300 4=301-400 5=401-500 6=501-600 7=601-700 8=701-800 9=801-900 10=901-1000 11=1001-1100 12=1101-1200 13=1201-1300 14=1301-1400 15=1401-1500 16=1501-1600 17=1601-1700 18=1701-1800 19=1801-1900 20=1901-2000 21=2001-2100 22=2101-2200 23=2201-2300

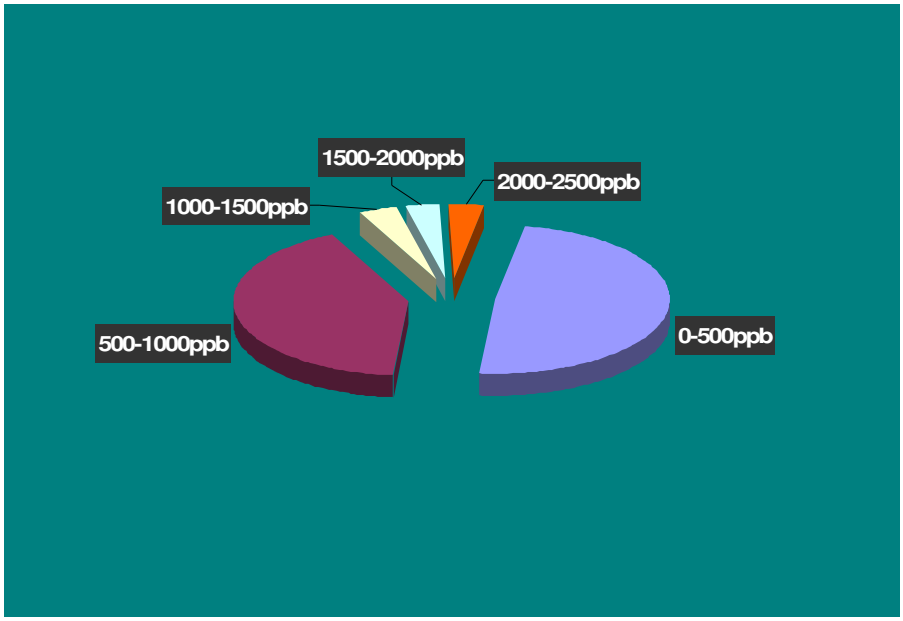


Figure 6: Pie explosion of AF cumulative percentages

DISCUSSION

Aflatoxin contamination of broiler feed commodities, and the natural aflatoxicosis in broilers have been reported worldwide, and also here in Iraq (15,16), but the occurrence of aflatoxin in poultry feed exhibits in most instances a geographical pattern, and Aspergillus species meat optimal conditions in tropical and subtropical regions, (16). Feed commodities likely to be contaminated with aflatoxins are many feed ingredients (17). Natural contamination of poultry feeds with aflatoxin was reported in many countries, like India (18), Malaysia(19), Indonesia (20), Sudan (21), Nigeria (22), Morocco(23), Poland(24), the United Kingdom(25) , Australia (26), and the United States(27), and recently here in Ninevah governorate (15).

The results showed that all broiler compound feed samples delivered from flocks with field aflatoxicosis had aflatoxin levels above 20 ppb, the regulatory levels in feeds in most countries. (Leeson, 1995).Aflatoxin levels were ranged from 22-2263 ppb, with a mean value of 592.7 ppb. The maximum AF level reported here was higher than that reported by us in the preceding work, in which mean AFB1 was 114 ppb, with a mean value of, and also higher than that reported in India of (11600ppb) (9), and in Indonesia of (6171ppb)(11), while it was higher than that reported in poultry feed in Nigeria by Shetty et al., (1987) of 1610ppb(10), and that in Egypt of 2000ppb (12). Most of the contaminated feed samples (89.9%) recovered in our study had AF concentrations between 22-1000 ppb, while the remaining AF levels of 1000-2500ppb were about 10% of all tested samples. These concentrations are very likely to induce field aflatoxicosis, since (28) reported that as little as little as 30 ppb was enough to induce field aflatoxicosis in broiler chickens. So it is very likely that AF concentration in tested mixed feed samples, could lead to many changes characteristics for aflatoxicosis in broiler flocks, which confirmed and resembled those reported by Rajion and Farrell (1976) (29), who found that feeding 1100 ppb of AF to New hampshire chickens were resulted in enlarged livers of necropsed chickens, reduction in body weight and poor feed conversion in the surviving birds. These adverse AF changes were

also reproduced by (Reddy *et al.,* 1984) (30), when fed broiler chickens AFB1 up to 1000 ppb for 28 days, or for 5 weeks by (Giambrone *et al.* 1985) (31), who stated that gross liver lesions indicative for aflatoxin toxicity, of yellow, ocher discoloration of the liver, with multifocal hemorrhages and white foci, accompanied by reduction in body weight gain and feed conversion occurred when AF was fed at concentration of 1000 ppb and more. These changes were also reported by (Doerr *et al.,* 1983) (32) by feeding AF at the same concentration for 7 weeks.

In addition to the effect of AF on broiler performance and immunity, it could deleteriously interact with different factors under field conditions. Some of these factors include presence of other mycotoxins in the feed like aflatoxin and ochratoxin A (43), aflatoxin and deoxynivalenol (44). Interaction of aflatoxin with other fungal infections like pulmonary aspergillosis has been reported in chickens(45). Interaction of AF with several dietary nutrients, like the change in response to AF with different source and level of dietary protein (46), or the greater effect of AF in broilers fed low fat diet (47), or the increased sensitivity of broilers to too small concentration of AF when fed diets deficient in riboflavin and cholecalciferol (48) has been reported.

It could concluded that as aflatoxin contamination of feeds is virtually inevitable, particularly in tropical and subtropical areas, like here in Iraq, where temperature and humidity favor development of Aspergillus fungi and their production of mycotoxins, several strategies should be developed in order to minimize the adverse effects of aflatoxins on poultry and also to prevent human aflatoxicosis. This could be accomplished by the most recent applied strategies like dietary supplements (49), detoxification of aflatoxin-contaminated feeds by physical and chemical methods(50)

REFERENCES

1. Peterson S W, Ito Y, Horn B W, Goto T. *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, A Nomius. Mycologia 2001; 93: 689 703.
2. Diener UL, Cole R J, Sanders T H, Payne GA , Lee LS, Klich MA. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annu Rev Phytopathol 1987; 25:249-270.
3. Gotty PJ, Bayman P, Egel DS, and Elias DS. Agriculture, aflatoxins and Aspergillus spp. 1-27. *in*: Powell A, Fenwick A, and Puberty J E (Eds) The genus *Aspergillus Plenum* Press. New York 1994.
4. Detory RW, Lillehoj EB, and Ciegler A. Aflatoxin and related compounds, p.3-178. In: A.Ciegler, Kadis S,and AJL S J (ed). Microbial toxins, vol, VI: fungal toxins. Academic Press, New York, N.Y. 1971.
5. Klich MA. Relation of plant water potential at flowering to subsequent cottonseed infection by *Aspergillus flavus*. Phytopathology 1987; 77:739-741.
6. Wilson DM, and Payne GA. Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops,p.309-325. *In* Eaton D L and groopman J D. (ed). The toxicology of aflatoxins , human health, veterinary and agricultural significance. Academic Press, San Diego, Calf 1994.
7. Davis ND, Diener U L. Some characteristics of toxigenic and nontoxigenic isolates of *Aspergillus flavus* and *A. parasiticus*. In: Diener UL, AsquithR L

and Dickens JW, EDS. Aflatoxin and *Aspergillus flavus* in corn. Southern Coop Series Bull.279, Craftmaster, Opelika, AL, 1983.
8. Calnek BW, Barness HJ, Beard CW, McDoougald LR, Saif YM. Disease of poultry.10th edition. Copyright. Iowa State University Press, Ames, Iowa U.S.A.1997.
9. Jindal N, Mahipal SK, and Mahajan NK. Occurrence of aflatoxin in compound poultry feeds in Haryana and effect of different storage conditions on its production. Indian J Anim Sci 1993; 63:71-73.
10. Shetty SN, Asuzu IU, Anika SM. Aflatoxin contamination of animal feedstuffs in Anambra State. Trop Vet 1987; 5: 21-25.
11. Purwoko HM, Hald B, Woistrup J. Aflatoxin content and number of fungi in poultry feedstuffs from Indonesia. Lett Appl Microbiol 1991; 12: 212-215.
12. Hegazy SM, Azzam A, Gabal MA. Interaction of naturally occurring aflatoxins in poultry feed and immunization against fowl cholera. Poult Sci 1991; 70: 2425-2428.
13. Shreef AM. Incidence of aflatoxins in broilers mixed feed in Ninevah governorate. Iraqi J V Sci 2007; 21:Under publication.
14- SPSS .Version 10. 2003.
15- AL-Sadi HI, Shareef AM, AL-Attar MY. Outbreaks of aflatoxicosis in broilers. Iraqi j.v.Sci 2000, 13: 93-106.
16- Christensen, CM, , Mirocha CJ, , Meronuck RA.. "Molds Mycotoxins andMycotoxicoses". Agricultural Experiment Station Miscellaneous Report 142. University of Minnesota, St. Paul, MN 1977.
17- Yoshizawa T. Natural occurrence of mycotoxin in small grain creals (wheat, barely, rye, oats, sorghum, millet, rice). In Smith JE and Henderson R (Eds). Mycotoxins and animal foods. CRC Press, BOCA Ratton, FL. pp.301-324.
18- Roa AG, Dehuri PK, Chand SC, Mishra Pk, Das BC. Aflatoxicosis in broiler chickens. Indian J Poult Sci 1985; 20: 240-244.
19- Abdulla AS, Lee OB. Aflatoxicosis in ducks. Kajian 1981; 44: 29-40.
20- Hetzel DJS, Hoffiman D, Van de ven J, Soeripm S. Mortality rate and liver histopathology in four breeds of ducks following long term exposure to low levels of aflatoxins. Singapor Vet J 1984; 8:6-10.
21- Dafalla R, Hassan YM, Adam SEI. Fatty and hemorrhagic liver and kidney. syndrome in breeding hens caused by aflatoxin B1 and heat stress in the Sudan. Vet Human Toxicol 1087; 29: 252-254.
22- Okoye JOA, Azuzu LU, Gugnani JC. Paralysis and lameness associated with aflatoxicosis in broilers. Avian Pathol 1988; 17: 731-734
23- Kichou K, Walser MM. The natural occurrence of aflatoxin B1 in Moroccan poultry feeds. Vet Human Toxicol 1993; 35:105-108.
24- Juszkiewicz T, Piskorska-Pliszczynska J. Occurrence of mycotoxins in animal feeds. J Environ Pathol Toxicol Oncol 1992; 11: 211-215.
25- Lamont MH. Cases of suspected mycotoxicosis as reported by veterinary investigation centers. Proc Mycotoxins Anim Dis 1979; 3: 38-39.
26- Bryden WL, lioyd AB, Cumming RB. Aflatoxin contamination of Austeralian animal feeds and suspected cases of mycotoxicosis. Aust Vet J 1980; 56: 176-180.
27- Hamilton PB. A natural and extremely severe occurrence of aflatoxicosis in laying hens. Poult Sci 1971; 50:1880-1882.

28- Jones FT, Huggler W, Hard HPB. Association of low levels of aflatoxin in feed with productivity losses in commercial broiler operations. Poult Sci 1982; 61: 861-868.
29- Rajion AM, Farrell DJ. Energy and nitrogen metabolism of diseased chickens; Aflatoxicosis. Br Poult Sci 1976;17: 79-92.
30- Reddy CV. Aflatoxins in feed:an enemy to poultry producers in the tropics. Misset Wld Poult 1992; 8:19-20.
31- Giambrone JJ , DINIER UL, Davis ND, Panagala VS, Hoerr FJ. Effects of purified aflatoxin on broiler chickens. Poult Sci 1985; 64: 852-858.
32- Doerr JA, Huff WE, Wabeck CJ, Chaloupka GW, May JD, Merkley JW. Effects of low level chronic aflatoxicosis in broiler chickens. Poult Sci 1983; 62:1971-1977.
33- Huff WE, Doerr JA. Synergism between aflatoxin and ochratoxin A in broiler chicks. Poult Sci 1981; 60: 550-555.
34- Huff WE, Kubena LF, Harvey RB, Hagler WM, Swanson SP , Phillips TD, Creger CR. Individual and combined effects of aflatoxin and deoxynivalenol(DON) in broiler chickens. Poult Sci 1986; 65:1291-1298.
35- Shoyinka SVO, Onyekweodiri EO. Clinico-pathology of interaction between aflatoxin and aspergillosis in chickens. Bull Anim Helt Prod Afr 1987; 35:47-51.
36- Diaz GJ, Squires EJ, and Julian RJ. Effect of selected dietary antioxidants on the incidence of fatty liver haemorrhagic syndrome in laying hens. Br Poult Sci 1994; 34: 621-630.
37- Hamilton PB, Tung HT, Harris JR, Gainer JH, Donaldson WE. The effect of dietary fat on aflatoxicosis in turkey. Poult Sci 1972; 51:165-170.
38- Hamilton PB, Tung HT, Wyatt RD, Donaldson WE. Interaction of dietary aflatoxin with some vitamin deficiencies. Poult Sci 1974; 53:871-877.
39- Ehrich M, Driscoll C, Larsen C. Ability of ethoxyquine and butylated hydroxytoluene to counteract deleterious effects of dietary aflatoxin in chicks.Avian Dis 1986; 30:802-807
40- Ibrahim IK, Shareef AM, AL-Joubory KMT. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res Vet Sci 2000; 69:119-22.