Efficiency Of Use Of Yeast Extract And Acetic Acid In Detoxifications Of Aflatoxin Contaminated Chicken Feeds.

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Abstract: One hundred samples of poultry feed, water, litter, throat and cloacal swabs (20 samples of each), were collected from diseased poultry farms at Giza Governorate and subjected for mycological examination. The results revealed that the isolated fungi represented 9 genera of moulds and one species of yeast. The most prevalent fungi in these samples was the genus *Aspergillus* (85%, 40%, 35%, 40% and 20%), respectively, which was at the top of all isolated fungi. The *A. flavus* was the most predominant isolated fungi from all collected samples (75%, 15%, 45%, 30% and 35%), respectively. Whereas, the results of aflatoxins detection in feed samples yielded a significant higher levels particularly aflatoxin B_1 which gave the highest mean level (160 ±3.50 ppb). On the other hand, the fungi of *A. flavus* and *A.parasiticus* which isolated from poultry feed samples produced aflatoxin B_1 at mean levels of (253 ±3.5 ppb and 165±4.8 ppb respectively) .Therefore, the control of aflatoxins contamination in feed became a critical demand. The experimental feeding of one day old Hubbard chicks was conducted including four groups(10 of each); the birds of group 1 was kept as a control and fed on uncontaminated basal diet; whereas the birds of group 2 fed on aflatoxin B_1 contaminated basal diet (2ppm) (aflatoxicated) ; but the chicks of group 3, was given a basal diet containing (3%) yeast extract treated contaminated diet with (2ppm) aflatoxin B_1; and group 4, was treated on basal diet having (3%) acetic acid treated aflatoxin contaminated (2ppm). The aflatoxicated group fed on AFB_1 without any treatment had significantly decreased in serum total protein, albumin,α2 ,β2, total globulins and cholesterol than control group. Also there is a significant increase in serum creatinine and enzymes activities of ALT, AST and ALP in aflatoxicated group. The treated aflatoxicated chicks by yeast extract (3%) and acetic acid (3%) showed a significant improvement in all altered biochemical parameters. The significance of the present results was fully discussed.

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Key words: yeast extract, acetic acid, detoxification, AFB_1& chicken feeds, protein electrophoresis and biochemical parameters.

1. Introduction:

Mycotoxicosis can be controlled by improving the methods of harvesting management, storing and transportation of feed in good environmental conditions. The frequent testing of feed for mycotoxins contamination should be adapted for the control of mycotoxins in the food chain (**Hassan *et al*., 2010 and 2011 and Abidin *et al.*, 2011).**

It was investigated that the potential production of aflatoxins by toxigenic fungi in feed reported as hazard to animal and poultry health which consumed these feeds (**Fraga *et al*., 2008 and Hassan *et al.*, 2009)**.

Aflatoxins (AFs) are ubiquitous in corn-based animal feed and causes hepatotoxic and hepatocarcinogenic effects. The most important aflatoxins in terms of toxic potency and occurrence was aflatoxin B_1. Poultry, especially chickens, are extremely sensitive to the toxic and carcinogenic action of AFB_1, resulting in economic annual losses to producers due to reduced growth rate, increased susceptibility to disease, reduced egg production and other adverse effects (**Rawal *et al.*, 2010 and Awad *et al.*, 2011)**. Hence, the strategies focus on the use of feed additives of natural and chemical origins to avoid the serious side effect of mycotoxins. However, certain feed additives have been successfully used to inhibit mold growth and to reduce the incidence of aflatoxicosis in animals. Nowadays, feed additives may added to poultry diets in very small quantities to improve the growth performance and economical efficiency. It is essential for biological functions of the animal including growth promoters, digestion, absorption, antimicrobial agent, metabolic modifiers probiotics and prophylactics **(Namur *et al*., 1998 and Hassan *et al.* , 2007 and 2008) .**The recent researches reported that biological decontamination of mycotoxins using microorganisms is one of the well known strategies for the management of mycotoxins in foods and feeds. Among the different potential decontaminating microorganism was *saccharomyces cerevisiae* represent unique, which are widely used in food fermentation and preservation. On the other hand the yeast creates a

starter culture for flour fermentation that has a stable feature of detoxication of aflatoxins **(Matur, 2010; Awad *et al.,* 2011 and Hassan *et al*., 2011).**

It was investigated that dry yeast is a feed supplement for livestock and poultry containing mannan oligosaccharides and Beta 1, 3-Beta 1,6D glucan. A naturally derived extracts from the cell wall of yeast (*Saccharomyces cerevisiae*), which is the most common species of yeast fed. It may be in a dried non formative form or an active dry yeast form. Nutritionist and feed manufactures can have the greatest impact on strengthening the animal immune system, (**Peppler and Stone, 1976; Awad *et al*., 2011 and Hassan *et al.,* 2011)**. Two components of the yeast cell wall that has greatest impact on poultry performance and immune system according to a number of researchers working in laboratories around the world are the phosphorylated mannan oligosaccharide protein (MOS) a protein modifier of gut microflora and Beta glucan. Glucans and mannans, the two main sugars, are present in about equal concentrations **(Reed and Nagodawithana, 1991)**. It is interesting to report here that **(Hashmi *et al*., 2006 and Awad *et al*., 2011)**, found that if (100 ml) yeast sludge contains (8.96 gm) yeast cells, it contains (0.26%) mannan oligosaccharide, which is the principal compound that bind the aflatoxins and ochratoxin A and improve the survival, yield and economics of commercial poultry production. While the yeast based mannan oligosaccharides enhanced poult performance as well as increasing immunoglobulin production **(Savage and Zakrzewska, 1996)**. Also, mannan oligosaccharide has been shown to counteract many of the badside effects of aflatoxin in poultry **(Stanley and Sefton, 1999).** On the other hand the previously toxicology and pathology studies showed that toxicity of AFB_1 was minimized by acidic treatment. The results indicated that aqueous acetic acid exhibits detoxifying activity in AFB_1 contaminated feeds and protects animals from chronic aflatoxin toxicity **(Youssef, 1990; Gazia *et al.*,1991 and Mendez *et al.*, 2007) . Pal *et al*., (2009)** stated that treatment of contaminated ration with acetic acid proved effective. Consequently, the diverse action and diseases resulted from fungal and mycotoxin contamination enforced the continuous trials of scientists to find out a new and safe method for their control .

So the aim of the present study is to evaluate the influence role of the feed additives with the use of yeast extract (3%) and acetic acid (3%) on improvement of biochemical parameters changes due to aflatoxicosis in broiler chicks.

2. Materials and Methods

1. Samples:

One hundred samples, including (poultry feed, water, litter, cloacal swabs and throat swabs) (20 of each) were collected from diseased poultry farms at Giza governorate. The birds in these farms suffering from a signs of aflatoxicosis including depression, diarrhea, loss appetite, decrease body weight gain and high mortality. The collected samples were transported to laboratory in clean sterile plastic page for mycological and mycotoxicological investigation.

2. Aflatoxins standard:

Standards of different types of aflatoxins and Immunoaffinity columns of these types were purchased from sigma chemical company (USA).

3. Antifungal:

As acetic acid and yeast extract (*Saccharomyces cerevisiae*) were purchased from **El Nasser** Pharmaceutical Chemical company.

4. Isolation and identification of moulds:

The collected samples were subjected for isolation and identification of fungi as recommended by **Conner *et al.*, (1992**).

5. Detection of aflatoxins in poultry feeds:

Fifty grams of the ground samples of feed were subjected for extraction and purification of aflatoxins using immune affinity column and quantitatively estimated by fluorometric method according to **Hansen, (1993**).

6. Production and estimation of aflatoxins (Gabal *et al.,* 1994):

The fungi of *A.flavus and A. parasiticus* that isolated from present feed samples were inoculated into flasks containing (50 ml) of sterile (2%)yeast extract solution containing (20%) sucrose medium. Inoculated flasks were incubated at (25ºC) for (7-10 days). At the end of the incubation period**,** extraction and detection of produced aflatoxins was estimated by fluorometric method as recommended by **Hansen (1993).**

7. Experimental animals:

Forty of apparently healthy broiler chicks of one day old were divided randomly into four groups, (each of 10 birds). Chicks of group 1 fed on a basal healthy diet free from aflatoxin and without any treatment and kept as a negative control. While, chicks of group 2 given the basal diet plus (2ppm aflatoxin B_1) **Harvey *et al.,* (1989)** and kept as positive control. Whereas, Chicks of groups 3 and 4 were treated with the AFB_1 (2ppm) diet supplemented with (3%) yeast extract and (3%) acetic acid, respectively .The experimental work was continues for a period of (3 weeks).

8. Biochemical investigations:

At the end of the experimental period, blood samples were collected from jugular vein from each bird using a disposable tuberculin syringes. Blood samples was taken without anticoagulant in centrifuge tubes, allowed to clot, and then centrifuged at (3000 rpm) for (10 minutes) for separation of serum which used to assay the biochemical parameters. Serum analysis included estimation of serum aspartate aminotransferase (AST) , alanine aminotransferase (ALT) and alkaline phosphatase (AlP) activities according to **Reitman and Frankel, (1957**) and **Belfield and Goldberg, (1971)** respectively, serum creatinine level according to **Ullmann and Bonitz, (1976) ,** serum cholesterol **Henry (1974)** ,total serum protein as described by**Pesce and Kaplan , (1978**) and serum protein fractions by polyacrylamide gel electrophoresis according to **Davis (1964)** and calculated according to SynGene S. No. 17292*14518 sme*mpcs**.**

9. Statistical analysis:

The obtained data were statistically analyzed using student's t-test according to **Petrie and Watson, (1999).**

3. Results and Discussion:

The ubiquitous mycotoxins often invade feedstuffs consumed by chickens causing mal performance, pathological alterations and metabolic disorders. Moreover, the danger from dietary aflatoxin is high in hot and humid climates.

The current data in (Table, 1) showed the isolation of nine genera of mould and one genus of yeast (candida). The most prevalent fungi in poultry feed, water, litter, throat swabs and cloacal swabs were the members of genus *Aspergillus* (85%, 40%, 35%, 40% and 20%), respectively, which was at the top of all isolated fungi. Other genera of mould

were recovered in different frequencies. Whereas, the yeasts of *Candida* sp. were isolated

in moderate frequency in all samples except poultry feed (30%, 35%, 30% and 25%) respectively. It is interesting to report here that species of *Aspergillus flavus* was the most frequent mould of *Aspergillus* species isolated from all tested samples (75%, 15%, 45%, 30% and 35%) respectively. While *A. parasiticus* recovered from samples of poultry feed litter and cloacal swab with the rate of (25%, 5% and 5%) The similar results were previously reported by **Hassan *et al.,* (2002) and Hassan and Mogda, (2003)** who recovered such species of fungi from poultry feeds, litters, water and internal organs in association with outbreak of mycoitoxicosis.

Other members of *Aspergillus* were isolated in various frequencies (Table, 2). The isolation of large numbers of fungi in present samples may be due to their exposure to environmental factors as high temperatures and humidity during harvesting, transportation, handling, processing and/or storage which help in all ways to fungal pollution by different genera of fungi. Poultry feed samples were evaluated for aflatoxins contamination. The current results in Table (3) showed that AFB_1 was detected in (80%) of samples of poultry feeds with maximum level of (1800 ppb); minimum level of (150 ppb) and with the mean level of (160±3.50). Whereas, AFB_2 was detected in (30%) of samples with maximum level of (2.0 ppb) ; minimum level of (0.5 ppb) and with the mean level of (1.50±0.10).On the other hand AFG_1 and AFG_2 were detected in (33.3% and 20%) of samples with maximum level of (14 and 3.9 ppb) ; minimum level of (5.5 and 0.6 ppb) and with the mean level of (9.70 ±1.71 and 2.25 ±0.01).Whenever, the maximal level allowed by Food and Drug Administration **(FDA)** is (20 ppb) for all feeds and foods **Schuller *et al.,* (1983**). The detected levels of AFB_1 in the present work were significantly higher and hazard for animal health.

On the other hand, the isolates of *A.flavus* (15) and *A. parasiticus* (5) that recovered from present feed poultry samples were screened for AFB_1 production on synthetic medium of YES. The obtained results yielded that all strains produced significant levels of toxin with a maximum level of (1235 and 220 ppb); with a minimum level of (30 and 110 ppb) and with the mean levels of (253 ± 3.5 and 165±4.8) respectively. Therefore, AFB_1 was the most prevalent form of aflatoxin used in this study (Table, 4).

The effects of dietary aflatoxin B_1 contamination on plasma enzyme activities were presented in (Table, 5). The results were shown in a significant increase in AST, ALT and ALP activities and increase in creatinine levels while decrease in cholesterol concentration. Aflatoxins are hepatotoxic in all vertebrate species and induce fat infiltration hepatocyte degeneration and necrosis and they alter the liver function **Riley and Pestka, (2005).** Also, this data could be results from damage of liver cell and bile duct obstruction due to proliferation of its cell **(Celik *et al.,* 2001)**.

Analysis of serum biochemical parameters (Table, 6) showed significant hypoproteinemia in chicks of (group 2) treated with AFB_1 (2ppm). These results were due to reduction in both albumin and globulin values. These changes may be due to that the metabolism of aflatoxin occurs in liver and cause reduction in protein synthesis and RNA production **Osuna and Edds, (1982)**. The aflatoxins inducing significantly decreased value in the results of A/G ratio. These findings were in agreement with

previous report **Basmacioglu *et al.*, (2005)** and were attributed to the liver toxicosis and/or to the inhibition of protein synthesis **Jindal *et al.*, (1994)**. The previous study added that α1, α2, β2 and γ1 were decrease while β1 and γ2 globulins were increased **(Kaneko *et al.*, 1997).** This finding may be due to impact of aflatoxin towered the hepatic cell and immune system **(Basmacioglu *et al.*, 2005).**

These results similar as reported by **Oguz *et al.*, (2002); Eraslan *et al.*, (2006);** and **Safameher, (2008)**. However, in treated chicks with aflatoxin and acidic acid/ or yeast extract (groups 3 and 4), the biochemical alteration in toxicated group were slightly improved by supplementation of feed with acetic acid or yeast extract. Where, there were a slight elevation of serum total protein, serum albumin globulins, cholesterol and conversely reduction of enzyme activities (ALT, AST and ALP) and creatinine compared to control group. The results indicated that the addition of (3%) yeast extract to aflatoxicated feed was most efficient in detoxification effect than addition of (3%) acetic acid, **(Abdo, 2004 and Yildirim *et al.*, 2011**) in chicken. Yeast contains mineral and vitamin like B-complex such these constituents are catalyst for oxidative enzyme of liver and decrease the adverse effects of aflatoxin. B-complex well restored the β-globulin which decreased by aflatoxin. **(Webster *et al.*, 1996 and Hassan and Mogda, 2003).** Also, yeast inhibits activity of cytochrom P-45o which activate the aflatoxin metabolite to epoxide ring. So it minimizes the immunosuppressive effect of aflatoxin **(Sengslag and Wurgler, 1994)**. On the other hand, **(Abdel-fattah *et al.*, 2008)** reported that total protein and albumin were not affected due to the inclusion of (1.5 or 3%) acetic acid in chicken fed during (42 days) period.

The presence of fungi and their toxins in feed, water and food reflected unhygienic measure during cultivation, irrigation harvesting transportation, handling, and storage and processing of feed and food. Therefore, frequent testing programs of food during different steps of production and routine cleaning of watering devices into which feed may be carried on birds is a precaution against mycotoxicosis which should not be overlooked before given to animals or human for consumption. This was increasing the quality of human food and animal's wealth.

Table (1): The prevalence of fungi in different examined samples:

Samples / Isolates	Poultry feed (20)		Water (20)		Litter (20)		Throat swabs (20)		Cloacal swabs (20)	
	No	%	No	%	No	%	No	%	No	%
Aspergillus. sp	17	85	8	40	7	35	8	40	4	20
Penicillium. sp	4	20	3	15	6	30	6	30	3	15
Mucor. sp	6	30	2	10	4	20	3	15	2	10
Rhizopus.sp	1	10	4	20	3	15	-	-	1	5
Fusarium.sp	-	-	1	5	2	10	-	-	1	5
Alternaria.sp	-	-	-	-	-	-	1	5	2	10
Cladosporium.sp	-	-	1	5	1	5	-	-	-	-
Curvularia. sp	-	-	-	-	1	5	-	-	1	5
Scopulariopss.sp	-	-	-	-	-	-	-	-	3	15
Candida.sp	-	-	6	30	7	35	6	30	5	5

Table (2): The identification of Aspergillus isolates from examined samples:

Samples / *Aspergillus* .spp	Poultry feed (20)		Water (20)		Litter (20)		Throat swab (20)		Cloacal swab (20)	
	No	%	No	%	No	%	No	%	No	%
A.flavus.	15	75	3	15	9	45	6	30	7	35
A.niger.	7	35	3	15	6	35	5	25	5	25
A.fumigatus.	8	40	2	10	5	25	1	5	4	20
A.candidus.	3	15	1	5	5	25	-	-	2	10
A.ochraceus.	2	10	1	5	2	10	1	5	2	10
A.parasiticus.	5	25	-	-	1	5	-	-	1	5
A.terreus.	1	5	1	5	-	-	-	-	1	5

%: Were calculated according to the No. of examined samples.

Table (3): Detection of aflatoxins present in the examined poultry feed samples:

Source of isolates	No. of Samples	+ ve samples		Levels of AFT ppb.		
		No.	%	Max.	Min.	Mean ± S.E.
AFB_1	15	12	80	1800	150	160 ±3.50
AFB_2	15	3	30	2	0.5	1.50± 0.10
AFG_1	15	5	33.3	14	5.5	9.70± 1.71
AFG_2	15	2	20	3.9	0.6	2.25 ± 0.01

Table (4): Aflatoxin B_1 production by isolated strains of *A. flavus* and *A. parasiticus* on synthetic medium (YEST) :

Tested isolates	Amount of $AFB_{1\,ug}$ / l of YES broth				
	Incidence		Levels of aflatoxins (ug/l of YES broth)		
	No. of +ve	%	Max.	Min.	Mean± S.E.
A.flavus(15)	15	100	1235	30	253 ±3.5
A.parasiticus(5)	5	100	220	110	165 ± 4.8

Table (5): Effect of yeast extract and acetic acid as detoxifier for aflatoxin B_1 on serum biochemistry: n=5

Groups / Prameters	Group 1	Group 2	Group 3	Group 4
AST(u/l)	126.20 ±1.14	148.90 ±1.53***	129.80±1.49	131.00±1.16**
ALT(u/l)	96.40 ±1.03	116.30 ±1.15***	106.20±1.02***	102.60±0.65***
ALP(u/l)	32.20 ±1.00	41.70 ±1.08***	35.40±1.11	37.60±1.03***
Cholesterol mg%	148.90 ±2.29	133.90±1.49	140.20±2.21	138.20 ±1.84
Creatinine mg%	1.13±0.29	1.53±0.06***	1.33±0.02	1.42±0.14

Results are expressed as mean ± S.E.

*$P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.00$

Group 1: Control. Group 2: treated AFB_1 (2ppm).

Group 3: treated AFB_1 (2ppm) +yeast extract (3%). Group 4: treated AFB_1 (2ppm) +acetic acid (3%).

Table (6): Patterns of protein electrophoresis in treated chicks sera: n=5

Groups / Parameters	**Group 1**	**Group 2**	**Group 3**	**Group4**
T.protein	4.92±0.13	4.36±0.15*	4.68±0.08	4.34±0.19
Albumin	1.32±0.07	1.13±0.09	1.25±0.16	1.20±0.10
Alpha 1	0.35±0.03	0.29±0.09	0.32±0.05	0.22±0.03
Alpha 2	0.45±0.01	0.30±0.06*	0.46±0.09	0.37±0.07
T-alpha	0.81±0.09	0.59±0.09	0.78±0.02	0.60±0.18
Beta 1	0.50±0.03	0.63±0.05	0.55±0.03	0.59±0.02
Beta 2	0.69±0.02	0.50±0.03**	0.60±0.06	0.55±0.05
T.beta	1.198±0.03	1.122±0.013*	1.151±0.023	1.135±0.017
Gamma 1	1.22±0.17	1.06±0.03	1.19±0.06	1.14±0.05
Gamma 2	0.37±0.04	0.46±0.02	0.31±0.08	0.28±0.01***
T.Gamma	1.59±0.04	1.15±0.03	1.50±0.01*	1.42±0.05
T.globulin	3.60±0.11	3.23±0.12*	3.43±0.10	3.15±0.13
A/G ratio	0.37±0.002	0.36±0.003***	0.36±0.001	0.38±0.10*

Results are expressed as mean ± S.E.

* $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$

Group 1: Control. Group 2: treated AFB_1 (2ppm).

Group 3: treated AFB_1 (2ppm) +yeast extract (3%). Group 4: treated AFB_1 (2ppm) +acetic acid (3%).

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